THE

AMERICAN NATURALIST

VOL. LX *July-August, 1926* NO. 669

VARIATIONS IN MAN AND THEIR EVOLUTIONARY SIGNIFICANCE[1]

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EVERY biological science is confronted constantly with the manifold problems of the variability of life. Although there exists an enormous literature on general variability and on particular individual variations, the true nature of variations, together with their underlying causes and possible effects, is still by no means thoroughly understood, and students in the different fields are as yet far from a universal agreement in regard to the ultimate interpretation of the available evidence. Some excellent theories or, better, working hypotheses, have been advanced in an attempt to explain and summarize the behavior of variations. These hypotheses, however, need to be still further tested by the findings in as many different branches of the natural sciences as possible.

In the following notes it is intended to enumerate some contributions by physical anthropology that have a direct bearing on the general biological questions of variability and that, indirectly, happen to connect the latter with some phases of evolution. Data resulting from the study of man himself are often apt to be interpreted in a more

[1] Address delivered November 17, 1925, at the Carnegie Institution of Washington, Washington, D. C.

or less biased manner; they can not be properly evaluated until they are compared with conditions in other primates. Furthermore, an examination of normal man alone can rarely rise above a mere recording of bare facts, but by contrasting human peculiarities with those of related animals, their full significance becomes at once apparent. Part of this paper, therefore, will be devoted to a brief discussion of some variations in monkeys and apes. Main emphasis will be placed on the presentation of the concrete results gained from the author's own investigations; the meaning of these facts must be restricted to a tentative sketching of the new and old theories which seem to be supported by these data.

What is a variation? A few introductory remarks in regard to this question may not be out of place here. A variation is the individual deviation from the typical condition of the race or species. It should, therefore, be a directly observable feature. However, the race or species is, strictly speaking, something abstract, a more or less arbitrary idealization of a group of individual organisms which resemble one another more than they do the individuals of another race or species. The characterization of the race or species is dependent upon the number of individuals observed, since it is supposed to represent that form which occurs most frequently among all the possible variations; in other words, the ideal type of a species or its representative should be the average from all the normal individuals of the species.

The above definition, like most definitions, expresses really very little of the true and complex character of the thing defined. Many new questions immediately suggest themselves, *e.g.*, do variations occur with equal frequency and intensity at different stages of growth and in different parts of the body? Are variations entirely dependent on heredity or largely due to environmental conditions? These, together with many more problems, can be answered, in part at least, by the following observations.

It is a well-known fact that among adults one finds individuals with long, slender hands and others with short,

broad hands. The latter type of hand has often been regarded as a consequence of hard manual labor. However,

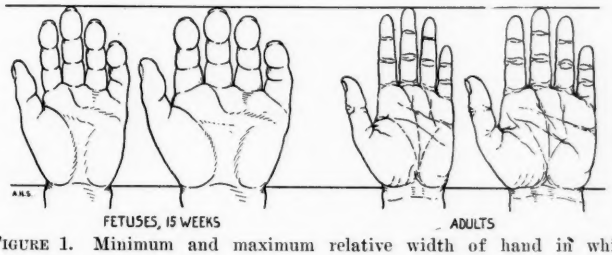

FIGURE 1. Minimum and maximum relative width of hand in white fetuses and adults. All reduced to the same hand length.

the two examples of human fetal hands in Figure 1 differ from each other more than do the two extremes of adult hands. In fetuses of fifteen weeks the hand width constitutes anywhere from 47 to 67 per cent. of the hand length —a spread over twenty units—whereas in adults these percentages range over only ten units, from 42 to 52 per cent. Some possible influence of long-continued intense labor on the shape of the hand is quite admissible, but such a causation could never account for all the variations found in the relative width of the hand. These are present long before the hand functions; indeed, hands vary to a greater extent before birth than when their growth is completed. A broad hand is therefore first of all a constitutional, congenital feature and is not acquired. It may, however, at times become accentuated through specialized function.

That such extreme variations as shown in Figure 1 are not isolated or pathological cases, but really constitute the normal limits of all observed variations, is demonstrated by the cephalic index (in Figure 2), which expresses the shape of the head. The width of the black polygons is determined by the range of variation of the cephalic index in the two age groups, the height by the number of cases in each index unit. These are normal distributions; that for fetuses is spread out more (75 to

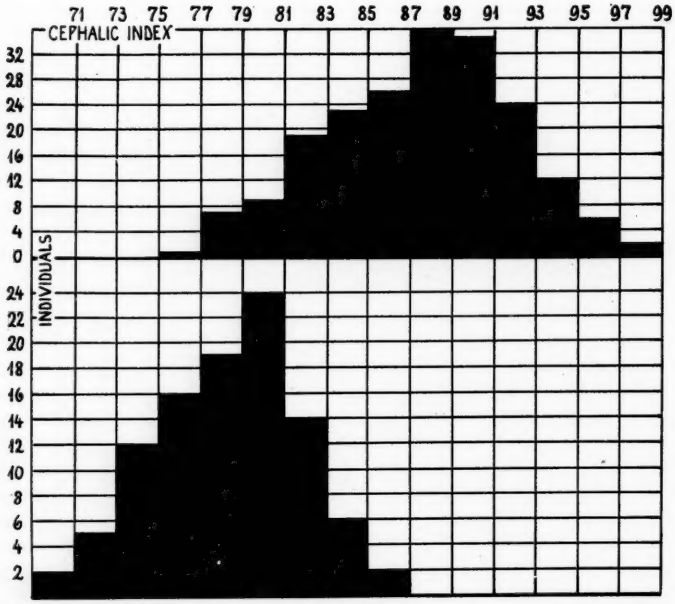

FIGURE 2. Frequency polygons for the cephalic index of two hundred white fetuses of the third and fourth month (upper figure) and of one hundred white adults (lower figure).

99) than that for adults (70 to 87), showing that this index is less variable in the latter than in the former. These polygons also demonstrate clearly that the extreme variations are linked together by an uninterrupted series of intermediate stages, which are the more numerous the closer they approach the average.

That pronounced individual characteristics develop very early in life is furthermore evident from the series of human fetal profiles in Figure 3. All degrees of underbite, edge-to-edge bite and over-bite are present early in intrauterine life. These different relations between the jaws are, therefore, not due to any faulty development of the teeth during childhood, as has been maintained, but are normal congenital variations, just as pronounced near the beginning as at the end of growth.

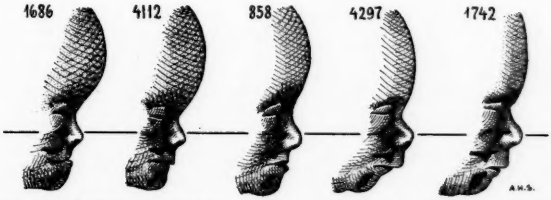

FIGURE 3. Different profiles of white fetuses, eleven to twenty-three weeks old, showing the various relations between the upper and lower jaw. The midsagittal sections of the jaws have been plotted in (solid black).

The two fetuses in Figure 4 are of the same race and sex and exactly the same age, yet the length of their

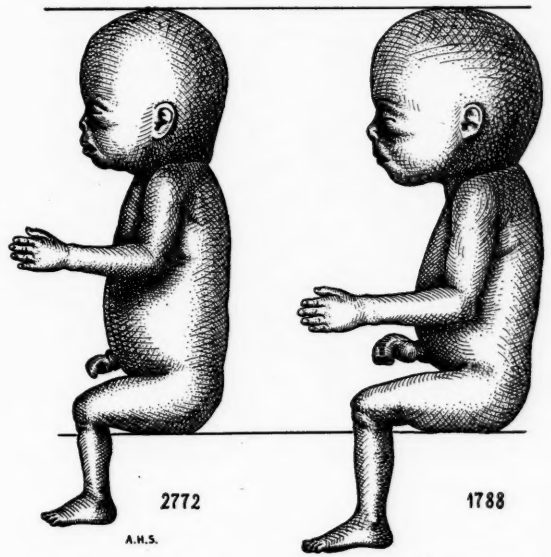

FIGURE 4. Minimum and maximum relative limb length in two male Negro fetuses, each with a sitting height of 143 mm.

limbs is strikingly different; in one the total length of the upper extremity amounts to 117 per cent. of the trunk height, in the other to 178 per cent. This difference, which is never surpassed by adults, indicates a decided lack of correlation between the size of the limbs and that

of the trunk. From these examples it would also seem more than likely that persons with relatively short limbs, as well as those with relatively long ones, must have possessed these distinctions long before they were born and did not develop them gradually in later life.

That all the proportions of the human body fluctuate as widely in early as in late growth stages is shown by

TABLE I

VARIATION COEFFICIENTS FOR THE MORE IMPORTANT BODY PROPORTIONS IN FETAL AND IN ADULT LIFE. THE COEFFICIENTS FOR FETUSES ARE AVERAGES OF THIRTY-TWO WEEKLY COEFFICIENTS

Proportion	Fetuses	Adults
Shoulder width: anterior trunk height	6.69	8.06
Hip width: anterior trunk height	7.29	6.18
Relative position of umbilicus	13.34	9.40
Total upper limb length: anterior trunk height	7.04	5.82
Forearm length: upper arm length	4.24	6.14
Total lower limb length: anterior trunk height	7.18	5.57
Leg length: thigh length	4.24	4.60
Total upper limb length: total lower limb length	3.03	2.75
Average head diameter: anterior trunk height	6.59	4.86
Head breadth: head length	4.45	4.31
Face breadth: upper face height	6.70	7.72
Nose breadth: nose height	7.77	7.76
Average variation coefficient	6.55	6.10

Table I, which is a compilation of the variation coefficients for some of the more important proportions obtained on many hundreds of fetuses and adults of the white race. It is sufficient here to state that a variation coefficient is a very sensitive measure of the variability of a character, taking into consideration every single case. The higher the coefficient the greater is the variability. The different proportions show widely differing degrees of variability and it is of special interest to note that, by and large, these degrees in a given proportion are about the same in fetal as in adult life. For example, in both age groups the percentage relation between the total length of the upper and that of the lower limb ex-

hibits the least variability, while the relative position of the umbilicus is the most variable character in both columns of Table I. The most important finding from this table is that the average coefficient of all the proportions is slightly higher in fetuses than in adults. This is ample evidence for the general statement that the human body is as variable in utero as in adult life; in other words, individual characteristics are as pronounced long before environmental factors have had any noteworthy chance to exert their influence as after the organism has been exposed to them for a lifetime.

It may be argued that the high variability of the fetal body is merely due to the rapidity of prenatal growth and that because of this it is to be expected that the different parts of the body would show little correlation with one another. Such an explanation, however, is entirely insufficient, as will become evident when the variation coefficients are compared with the growth increments, as is done for one example in Table II. By far

TABLE II

RATE OF GROWTH OF ANTERIOR TRUNK HEIGHT AND SHOULDER WIDTH (INCREASE WITHIN A WEEK EXPRESSED IN PERCENTAGE OF SIZE AT BEGINNING OF WEEK) AND VARIABILITY OF PROPORTION BETWEEN THESE TWO MEASUREMENTS IN WHITE FETUSES AND ADULTS

	Fetal month:	3rd	4th	5th	6th	7th	8th	9th	10th	Ad.
Average weekly increment:	Ant. trunk height	29.2	18.0	9.5	8.2	5.7	5.0	1.6	5.4	0
	Shoulder width	24.3	19.3	11.1	6.2	4.8	3.5	2.4	6.2	0
Average weekly variation coefficient	$\frac{\text{Shoulder w.}}{\text{Trunk height}} \times 100$	6.5	8.0	9.6	8.7	7.3	3.3	4.3	5.9	8.1

the most rapid growth occurs during the third month, when the trunk height, for instance, increases in size per week nearly 30 per cent. of its size at the beginning of the week. This increment diminishes very markedly, reaching a minimum of 1.6 per cent. in the ninth month. Yet this tremendous decrease in growth rate is not at all paralleled by a corresponding decrease in variability;

the latter seems rather to be completely independent of the rate of growth.

Not only does each individual differ from all other individuals from the earliest stages of growth, so that every person at any age is really a variation, but the products of the development of the two halves of a single fertilized ovum also vary to a certain degree. They are never exactly alike, from the embryonic to the adult stage. This fact shows itself in two ways, by asymmetries and by the differences between single-ovum twins.

TABLE III

PERCENTAGE FREQUENCIES OF SYMMETRY AND ASYMMETRIES AND AVERAGE DIFFERENCES (INCLUDING CASES OF SYMMETRY) BETWEEN MEASUREMENTS ON THE TWO SIDES (EXPRESSED IN PERCENTAGE OF SMALLER MEASUREMENT) IN FETUSES AND ADULTS[2]

Measurement	Age	Cases	r. > l.	r. = l.	r. < l.	Average % difference
Humerus length	fetal	100	52	27	21	1.3
Humerus length	adult	105	54	24	22	1.5
Foot length	fetal	100	33	20	47	1.4
Foot length	adult	500	31	16	53	1.1
Ear height	fetal	100	39	25	36	2.9
Ear height	adult	100	33	37	30	1.9

Table III compares the asymmetries in the length of the upper arm bone, in the length of the foot and in the physiognomic ear height of fetuses with the corresponding asymmetries in adults. The difference between these measurements on the two sides of the body range from 0 to 13.3 per cent. (of the smaller measurement) in case of the ear height, to 7.7 per cent. in case of the foot length, and to 7.5 per cent. in case of the humerus length; *i.e.*, the degree of asymmetry varies considerably. In

[2] The fetuses are all of the white race and range in age from the fourth month to birth. All the adults are whites, except fifty-four Negroes included in the series for humerus length. Detailed data on the humeri, used in the latter series, were published by the author in 1918 (*Amer. Jour. Anat.*, Vol. 23, pp. 155–173). For the measurements on 438 of the 500 adult whites, used in the series on foot length, the author is indebted to Dr. R. B. Bean.

fetuses, as in adults, the humerus is longer on the right side in over half the cases. This early occurrence of asymmetries in arm length proves that such asymmetries in the adult are rarely, if ever, due to any specialized function, such as right-handedness. Even though the majority of persons use the right arm by preference and may thus give it a greater growth stimulus, one would have to resort to the assumption of the inheritance of an acquired character to explain the prenatal existence of this asymmetry. That this can not be maintained, however, is evident from the finding that in apes, monkeys and even turtles the two humeri are found with great regularity to be of unequal length.

Asymmetries in the foot, in contrast to those in the upper arm, favor the left side, but, like those in the latter, they are as pronounced in fetal as in adult life. Asymmetries in the size of the outer ear are very common in man and apparently even more common in fetal than in adult life. In the ear size there exists no preference, as in the first two measurements, of one side over the other. The ears of monkeys are as asymmetrical as those of man. The author has examined specimens in which the ears differed from each other to an extent of over 10 per cent. of the size of the smaller ear. No one will doubt that these asymmetries exist entirely independent of environmental influences; they are purely congenital variations in the two sides of the body.

Directly comparable to asymmetries are the differences between so-called identical twins. This type of twins is derived from a single ovum; the two individuals must theoretically contain, therefore, the same hereditary elements. That, in spite of this, these twins are never really identical, but show differences even greater than most asymmetries, is due to the independent variability in the products of the two halves of the ovum, which is the same explanation as that for asymmetries.

Table IV shows, by the example of the cephalic index, that single-ovum twins can be as different in a given

TABLE IV

CEPHALIC INDICES (HEAD WIDTH—HEAD LENGTH RATIO) IN SINGLE-OVUM TWINS OF DIFFERENT AGES

Age	Twin A	Twin B	Difference
5 months fetal life	77.4	83.1	5.7
8 months fetal life	78.2	81.5	3.3
2 years 4 months	79.4	82.7	3.3
12 years	79.2	83.9	4.7
14 years	80.5	84.2	3.7
22 years	69.5	72.2	2.7

bodily proportion in fetal as in adult life. The shape of the head, for instance, does not become modified in the course of postnatal life through differing conditions to which the two individuals may have been submitted; their heads showed a difference in shape in early intrauterine growth stages, representing congenital variations which

TABLE V

AVERAGE PERCENTAGE DIFFERENCES BETWEEN HEAD MEASUREMENTS OF TWINS OF DIFFERENT AGES[3]

Age	Single ovum	Undetermined	Double ovum
6 months fetal life	3.89	3.49	.....
7 months fetal life	.....	6.85	.....
8 months fetal life	3.07	5.34	.....
8 months fetal life	6.28	.....	.....
Newborn	2.91	.....	.....
4 years	.....	.....	3.71
4 years	.....	.....	3.33
5 years	.....	.....	4.44
6 years	4.11	.....	1.50
8 years	3.11	.....	.....
12 years	2.48	.....	.....
14 years	1.82	.....	.....
22 years	2.15	2.68	.....
24 years	.....	2.04	.....

[3] The figures in this table were obtained according to the formula:

$$\left(\Sigma \frac{mA - mB}{\frac{1}{2}(mA + mB)} \times 100\right) : n.$$

mA stands for measurement on twin A, mB for the same measurement on twin B, n signifies the number of measurements taken on each twin, in this case 19.

are in principle the same as asymmetries. This fact is still more clearly brought out by Table V, which lists the averages of the percentage differences in the more important head measurements. The first column contains the figures for single-ovum twins; the second for twins which were most likely of this type, but as to which no definite record exists; the third column gives figures for twins which were derived from two separate ova. It is rather surprising that apparently single-ovum twins resemble each other to no greater degree than do double-ovum twins. However, this degree varies a great deal; in one set of twins the corresponding measurements differ on an average only 1.5 per cent., while in another set they may differ nearly 7 per cent. One definite conclusion, and the most important one, to be drawn from this table, is that the degree of resemblance between twins, no matter of which type, does not diminish with age. Here again it is impossible to demonstrate any influence of postnatal environmental factors on human variations. All the forms and degrees of the latter have been found at stages in development before outer conditions could be held responsible.

Anthropological literature contains many statements to the effect that human variability is unique in its intensity, and that the only other variability that approaches it is that found in domesticated animals. It is argued that in the latter, as in civilized man, sexual selection and selective mortality have largely disappeared as standardizing factors. It is also frequently taken for granted that modern man varies so markedly because of his widely differing diets, occupations, diseases, geographical environment, etc. In short, there exists among writers familiar with human variability alone a tendency toward the more or less vague belief that the degree and frequency of human variations is largely determined by conditions outside of the body, or at least outside of the germplasm. There is available sufficient proof for admitting that the above-named factors can at times have

a marked influence on the human body, but from the findings on variations in the embryo the author is forced to the conclusion that the extent of these influences has been greatly overestimated. In order to test the correctness of this theory further and by more direct evidence, the author collected large series of monkeys in their natural habitat. Two species, a Spider monkey and a Howler monkey, were obtained within strictly limited areas. These primates lived under ideally uniform environmental conditions; the individuals of each series had, without exception, the same diet, the same occupation or exercise, the same climatic conditions and even the same kind of ancestors. Here, if anywhere, we might expect a low variability.

A careful analysis of this material reveals the fact that the variability of wild monkeys is fully as great as that of modern civilized man. The proportions in Howler monkeys of different ages, examples of which are compiled in Table VI, show individual fluctuations which equal any human variations; *e.g.*, the total length of the

TABLE VI

SOME BODY PROPORTIONS (TOTAL UPPER AND LOWER LIMB LENGTH AND HEAD MODULE IN PERCENTAGE OF ANTERIOR TRUNK LENGTH AND LENGTH OF TAIL IN PERCENTAGE OF PRAECAUDAL SPINE LENGTH) IN HOWLER MONKEYS (ALOUATTA PALLIATA) OF FETAL, NEWBORN AND ADULT GROWTH STAGES

Sitting height: (mm.)	Upper limb l.: trunk length	Lower limb l.: trunk length	Aver. head diam.: trunk length	Tail length: praecaudal spine
135 (fetus)	182.0	127.1	58.1	144.6
137 (fetus)	162.2	118.5	54.6	131.5
168 (newborn)	181.4	131.4	54.3	181.9
169 (newborn)	171.3	127.7	48.9	191.1
170 (newborn)	161.3	115.6	49.1	180.6
442 (adult, ♂)	163.7	125.2	26.6	153.2
444 (adult, ♂)	151.2	121.5	27.1	135.8

upper extremity amounts in one adult to 151 per cent. of the trunk length, in the other adult to 164 per cent. As in man, these variations are just as pronounced in fetal as in adult life.

The two extreme variations in the facial profiles of Howlers, shown in Figure 5, are not isolated cases, but

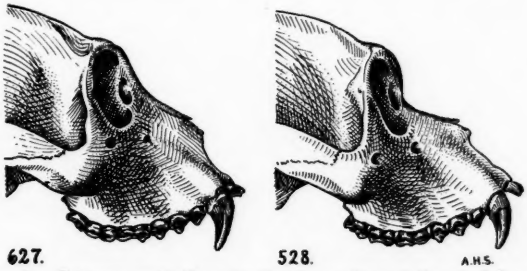

FIGURE 5. Extreme variations in the formation of the facial profile of the Howler monkey (Alouatta palliata, ♂, ad.).

merely the ends of the author's series when grouped according to this feature. The gap between these extremes could easily be bridged by all the intermediate variations. It is not surprising to encounter men with "Roman" noses and men with "pug" noses, living side by side in a city, but to find both these nasal types in monkeys of a kind in a small area of jungle is rather unexpected.

One of the most interesting variations in the Spider monkey lies in the different development of the forehead. Cases in which one can speak of a real forehead, in the sense in which the term is applied to man, are not rare. An extreme example of this is shown in the left picture of

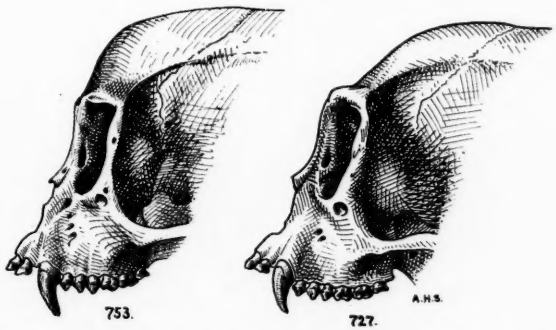

FIGURE 6. Extreme variations in the formation of the forehead of the Spider monkey (Ateles Geoffroyi, ♂, ad.).

Figure 6. Within the same troupe of monkeys, however, there occurred also individuals with low retreating foreheads such as that pictured on the right of Figure 6. These two monkeys were of the same sex and of approximately the same age; if found by themselves, without the intermediate, connecting degrees, they might easily have led a taxonomist to propose new subspecies. These two figures show strikingly how widely normal variations may fluctuate without our being able to say that they are due either to different environmental factors or to any particular form of selection.

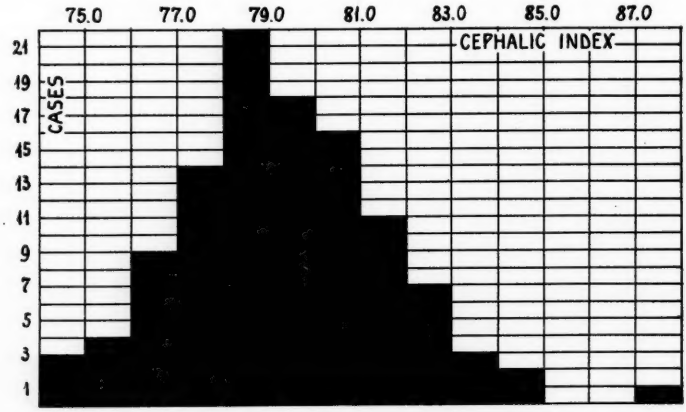

FIGURE 7. Frequency polygon for the cephalic index of 110 adult Howler monkey skulls (Alouatta palliata).

The frequency polygon in Figure 7 illustrates the normal distribution of variations in these primates, as exemplified by the cephalic index in Howler monkeys. A still larger series of cases would, no doubt, have connected the isolated high index on the extreme right with the rest of variations. The average of this proportion is 79.5, but it may be as low as 74, or as high as 88, a range which even surpasses that of many groups of man.

The degree of variability in these monkeys is most accurately compared with that in man by means of the variation coefficients. This is done in some skull measure-

TABLE VII

VARIATION COEFFICIENTS [(C ♂ + C ♀) : 2] FOR SOME SKULL MEASUREMENTS AND INDICES[4] OF ADULT HOWLER AND SPIDER MONKEYS COMPARED WITH THE CORRESPONDING COEFFICIENTS OF SOME HUMAN RACES[5]

	Length	Width	Ceph. index	Base index	Face index
Alouatta palliata	2.90	3.00	2.89	2.98	3.93
Ateles Geoffroyi	3.47	3.46	4.07	3.21	5.46
Swiss Alpines	.....	.....	3.08	3.67	5.25
Bavarians	3.47	3.64	3.79	.....	.....
Greenland Eskimos	.....	.....	.....	3.05	.....
Melanesians	2.97	3.04	.....	.....	.....

ments and indices in Table VII. First of all, it is apparent from the two uppermost rows of figures that the Spider monkey is in every point somewhat more variable than the Howler monkey, indicating that some species may possess a greater inborn tendency to deviate from the type than some other species, living under exactly the same conditions. Furthermore, the table shows that the coefficients for the human groups are practically no greater than those for the monkeys, particularly the "Spider" species. These data are merely samples, picked at random from a much greater mass of evidence, all tending to show that even under the most uniform environmental conditions, and certainly without domestication, a group of primates may possess as high a variability as modern man.

All the facts thus far presented indicate that every part of the body of every individual varies at every stage

[4] Length: greatest length from supraglabellare to occiput. Width: greatest width of brain case (over parietals or squamae temporales). Cephalic index: width in percentage of length. Base index: biauricular width in percentage of basion-nasion distance. Face index: face height (nasion-prosthion) in percentage of bizygomatic width.

[5] The coefficients for man are quoted from R. Martin (Lehrb. d. Anthrop., 1914); M. Reicher (Untersuchungen über die Schädelform . . . , Zeitschr. Morphol. u. Anthropol., Vols. 15–16, 1912); A. H. Ried (Beiträge zur Kraniologie . . . , Beitr. z. Anthrop. u. Urgesch. Bayerns, Vol. 18, 1911); A. H. Schultz (Anthropologische Untersuchungen . . . , Arch. f. Anthrop., N. F., Vol. 16, 1917).

of growth. These variations originate of their own accord, so to speak, at the beginning of development. The effects of conditions outside of the organism may superimpose themselves at times upon these normal congenital variations, but it would seem that this influence does not play as important a rôle as has often been assumed. Many, if not most, variations tend to be inherited, but it is difficult to explain on that basis alone why these monkeys, with their unmixed ancestry, are so variable, or why single-ovum twins are never identical. These rather involved questions can not be discussed in further detail here; they would carry one entirely into the field of genetics. The second part of this paper will be devoted instead to remarks on the relation between variability and evolution.

Evolution without variations is unthinkable. Changes that are potentially evolutionary are constantly occurring in the form of individual variations. When such variations happen not to be disadvantageous (so that they will not be eliminated again through selective mortality) they may become more numerous through inheritance and, indirectly, through the discarding of the opposite unfavorable variations. They then will actually be able to influence the species as a whole by shifting the average, or ideal type of the species, in the direction of their deviation from the original type. This is partly a mathematical consideration and could easily be proved by simple arithmetic. Organisms, or parts thereof, which do not vary can not change or evolve in successive generations. On the other hand, such animals or such body parts as vary the most are capable of undergoing the most rapid and marked evolutionary changes. By expressing this statement in a slightly different way it furnishes a helpful suggestion for diagnosing in a general way the relative specialization of a given structure in a species: The most extensive changes have taken place (or are under way) in those bodily parts that are the most variable, whereas the structures that fluctuate but little

in various individuals have deviated (or are deviating) to no great extent from their ancestral condition. This rule can, of course, hold true only in general. Here, as everywhere else in nature, there are at work many other factors which interfere with the exclusive correlation between two processes—variability and evolution. However, even in limiting this theory to its most conservative form, the assertion must stand that, wherever a bodily structure is found to be markedly variable, the suspicion is fully justified that this part has become different and specialized through evolution. Its condition in the species is still undergoing change; it has not yet definitely settled down, so to speak, as manifested by its variations, nor reached a uniform stage of perfection in all individ-

TABLE VIII

VARIATIONS IN THE NUMBER OF VERTEBRAE COMPOSING THE VARIOUS REGIONS OF THE SPINE OF ORANG-UTAN (FIFTEEN CASES FROM REPORTS IN THE LITERATURE AND TWENTY-FOUR CASES FROM THE AUTHOR'S OWN OBSERVATIONS)

Region of Spine	Number of Vertebrae	Per Cent. of Cases
Cervical	7	100
Thoracic	12½	5
	12	67
	11½	5
	11	23
Lumbar	5	13
	4½	3
	4	72
	3½	8
	3	4
Sacral	6	6
	5	80
	4	14
Coccygeal	5	3
	4	19
	3	42
	2	30
	1	6

uals. These theoretical speculations can be made clearer by some concrete examples appertaining to man and other primates.

Table VIII compares the variations in the number of vertebrae in the different regions of the spinal column of orang-utans. In all the specimens of this ape examined the cervical region contained seven vertebrae; *i.e.*, 100 per cent. of the cases were true to the type. In the thoracic region two thirds of the animals had twelve segments, which must hence be regarded as the typical number; the remaining 33 per cent. deviated in both directions from the norm, the variations affecting at times only one side of the body. For the lumbar region the average of four vertebrae was found in 72 per cent. In the sacral portion of the spine only one fifth of the specimens showed numerical variations. In the coccygeal region, finally, 58 per cent. of the entire material was different from the norm. While these figures, based upon a small series only, are far from definite, they nevertheless indicate with certainty a most significant difference in variability in the different spinal portions. For instance, there exists a striking contrast between the cervical region, with its absolute constancy in the number of vertebrae, and the caudal region, in which over half the cases are numerical variations. According to the theory outlined above, we must suspect a marked phylogenetic change in the latter region and the original unaltered condition in the former. In all mammals, with the exception of a few highly specialized forms, which have no connection with the phylogeny of primates, there are seven neck vertebrae. The cervical spine of orang has not, therefore, changed in evolution. In contrast to this, a long tail, composed of a very considerable number of vertebrae, is the rule for the lower primates; there can be no doubt, therefore, that the tail of orang has become greatly reduced in the course of its evolution. This reduction has gone even farther than in man, whose coccyx still consists, on an average, of four or five segments. The present species of orang has to be defined as pos-

sessing three coccygeal vertebrae, but 30 per cent. of orangs have only two, and 6 per cent. only one. Hence, in thirty-six out of every one hundred orangs the tail has undergone a greater reduction than is typical for the species, while in twenty-two others the typical reduction is not yet attained, these still having four or five segments. In other words, an individual or a structure may show acceleration or retardation in comparison with the state of evolution of its species as a whole. Such variations represent different steps in the direction in which a given structure is being changed.

In both man and orang the trunk vertebrae above the pelvis have become reduced in number during evolution. This reduction, like that of the tail, has been more ex-

TABLE IX

VARIATIONS IN THE NUMBER OF THORACICO-LUMBAR VERTEBRAE IN ORANG-UTAN AND IN MAN[6]

Number of thoracico-lumbar vertebrae:		18	17	16	15
Per cent. of cases	Orang-utan (81 cases)	.....	14	74	12
	Man (748 cases)	3	96	1	.....

treme in orang than in man. As shown in Table IX, the former has, as a rule, only sixteen of these vertebrae, while man still has seventeen. It must be mentioned here that orang has the lowest number of any primate and that all the monkeys, of both the Old and the New World, have more of these vertebrae than man. This greater evolutionary change in orang is associated with a higher variability in this character than is found in man. In the latter only 4 per cent. of the cases deviate from the normal number, in the former 26 per cent.

The phylogenetic reduction in the number of trunk segments in orang has affected also the front of the thorax. The breast bones, pictured in Figure 8, illustrate their

[6] These percentages are calculated from figures quoted by T. W. Todd (*Anatom. Record*, Vol. 24, 1922, p. 282). In addition, use was made of data on thirty-one orangs, examined by the author or described in such literature as not previously included by Todd.

very marked variability, which accompanies the phylogenetic shortening of the sternum. Not only do the various parts of the corpus sterni ossify very irregularly, but the number of ribs attached to the sternum varies from only twelve to as many as sixteen. Similar variations occur in man, but are not as frequent as in the orang. In both these primates, however, the reduction takes place mainly at the lower end of the thorax. This is evident from the following observations. Elimination of the first pair of ribs (resulting in eight cervical vertebrae) and a seventh spinal segment bearing ribs are exceedingly rare

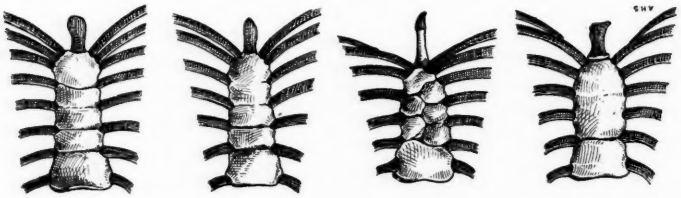

FIGURE 8. Variations in the sternum and in the number of sternal ribs in adult orang-utans. From left to right: twelve sternal ribs (total number of ribs twenty-two); thirteen sternal ribs (total number twenty-two); fourteen sternal ribs (total twenty-four); sixteen sternal ribs (total twenty-five).

findings, whereas eleven to thirteen pairs of ribs and variations in the size of the last pair are of common occurrence and well known in man. Furthermore, the lower end of the sternum, particularly the xyphoid process, is much more variable than the upper end, the manubrium.

In more or less isolated primate forms the limbs have become extremely lengthened in the course of perhaps relatively recent evolution. The Spider monkeys among the platyrrhines and the gibbons among the catarrhines show by far the greatest proportionate length in the upper extremities. On the other hand, while these two specialized groups show also considerable lengthening of the lower limbs, they are surpassed in this respect by man, who is characterized by a relative lower limb length unequaled in any other primate. Figure 9 demonstrates

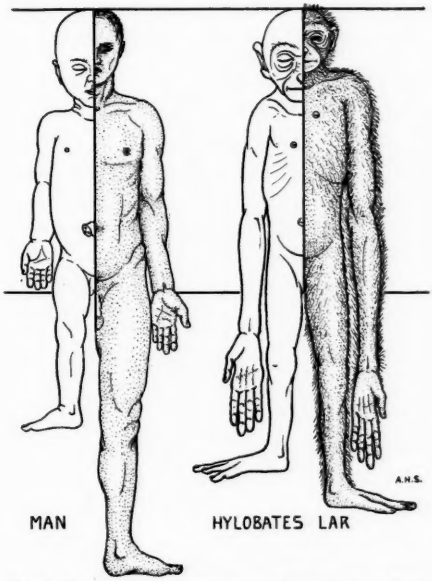

FIGURE 9. Semi-diagrammatic representation of the exact body proportions of new-born (left halves) and adult (right, dotted halves) man and gibbon. All reduced to the same sitting height.

that in the gibbon the adult proportions in limb length have been almost attained at birth. Man, in contrast to this, reaches his extreme specialization in lower limb length only during postnatal growth. Ontogenetically, therefore, the changes in the lower extremity of man are slower and occur later than those in the upper limb of the gibbon. Theoretically, these changes have become possible only through an evolutionary increase in the growth capacity of the limbs. This increase does not affect all individuals to the same degree, so that the newly changed character has a marked tendency for variations. That the arm of the gibbon (or its close relative, the Siamang) and the leg of man are really more variable than limbs of moderate length is shown by Table X. In adult white man the total length of the upper extremity constitutes on an average 153 per cent. of the anterior trunk height,

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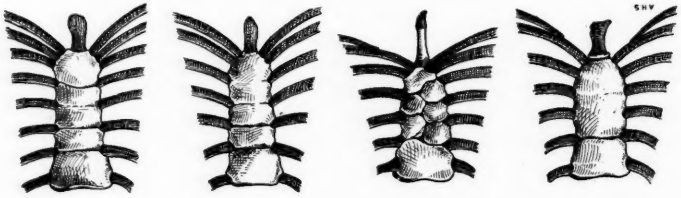

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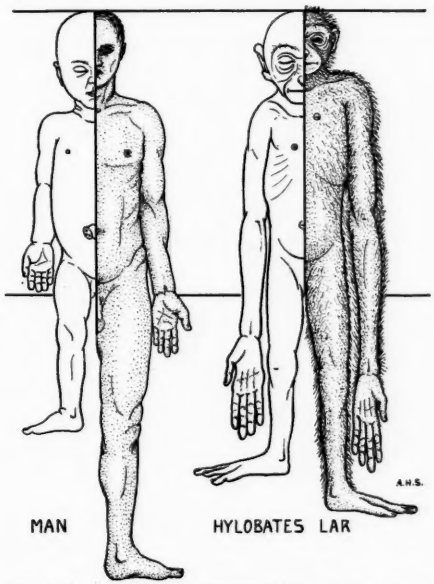

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TABLE X

RELATIVE LENGTHS OF THE UPPER LIMBS OF MAN AND SIAMANG APE AND OF THE LOWER LIMBS OF MAN AND PATAS MONKEY TOGETHER WITH THE VARIATION COEFFICIENTS OF THE PROPORTIONS IN THESE LIMBS.[7]

Upper Extremity			Lower Extremity		
Proportion:	White men	Siamang	Proportion:	White men	Patas monkey
Total upp. limb l. / Trunk height	152.7	246.9	Thigh + leg / Trunk height	158.5	99.7
	Variation coefficient			Variation coefficient	
Upper arm+forearm / Trunk height	5.04	8.36	Thigh + leg / Trunk height	5.43	4.89
Upper arm length / Trunk height	5.41	7.97	Thigh length / Trunk height	5.28	5.35
Forearm length / Trunk height	5.23	8.34	Leg length / Trunk height	6.20	5.37
Hand length / Trunk height	5.18	9.76	Leg length / Thigh length	4.76	4.70
Forearm length / Upper arm length	3.56	3.67			
Hand length / Forearm length	3.95	7.85			
Average coefficient	4.74	7.66	Av. coefficient	5.42	5.08

a proportion which stands close to that of most lower monkeys. In the Siamang, on the other hand, this relative upper limb length amounts to 247 per cent. The average variation coefficient for the proportions of the upper limb is in man 4.74, in the Siamang 7.66; *i.e.*, the arm of Symphalangus, which has evolved to two and a half times the length of the trunk, is subject to much greater variation than the arm of man, which has remained at a length of only one and a half times that of

[7] This table is based upon data contained in the paper by T. Mollison (Die Körperproportionen der Primaten. Morphol. Jahrb., Vol. 42, 1910). The series of white men comprises one hundred adults, that of Siamangs (Symphalangus syndactylus) twenty-nine adult specimens, and that of Patas monkeys (Erythrocebus patas) twelve adult specimens.

the trunk. In comparing, on the other hand, the long lower limb of man with that of a monkey, in which this limb is proportionately short, it is found that man shows the higher variability (see right side of Table X). It is interesting to learn also from Table X that in man the average variation coefficient for the lower extremity is higher than that of the upper extremity, since the latter has undergone much less change in evolution than the former. The much higher variability in the long arm of the Siamang than in the long leg of man can perhaps be interpreted as indicating that the human leg has become stabilized and has reached a definite goal in its evolutionary lengthening, whereas the arm of the ape has not yet reached its ultimate size. However, as mentioned before, there are so many other factors which might influence these relations that no absolutely certain conclusions can be drawn from these observations alone: a great relative limb length may be partly due to a phylogenetic shortening of the trunk; the ontogenetic duration of the increase in limb length may influence the variability; and, finally, there may be, and most likely is, a difference in general variability in different primate species.

Besides evolutionary changes resulting in an addition to some bodily part, such as limb length, there have also occurred many other changes which have brought about a decrease in some previously well-developed structure. That this is also accompanied by marked variability in the affected part will be shown by the following examples.

The thumb has become reduced in many primates; indeed, in a few this digit is entirely eliminated from the outer hand. In the Spider monkey, for instance, there is normally no trace of it left; upon dissecting the hand, however, one finds the rudimentary metacarpus for the thumb, which varies in its degree of reduction from an almost complete absence to a bone of very considerable length and thickness. Furthermore, in two out of fifty-eight hands (Ateles Geoffroyi) which the author was able to examine, there was still a small outer thumb, contain-

ing rudimentary fused phalanges. The high variability in this degenerated thumb forms a striking contrast to the remarkable constancy of the human thumb, which is the least reduced of any primate.

Similar conditions prevail in other digits. In the orang-utan, for instance, the great toe has, comparatively

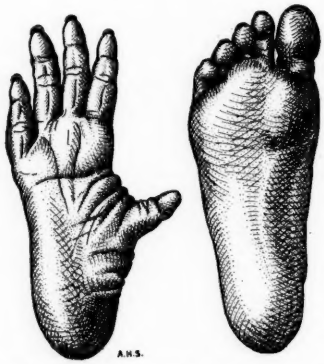


FIGURE 10. Foot of adult orang-utan (left) and of adult man (right).

speaking, degenerated. As shown by Figure 10, this toe is proportionately short and weak; it is certainly much less developed than in any of the other apes. In the majority of orangs it bears either no nail at all or merely a small trace of one, and in about every second case the two normal phalanges of the hallux are fused into one.

As high a variability as is characteristic for the great toe of orang exists in the little toe of man, which shows undoubtedly a tendency to become rudimentary. The nail of this toe varies much more than any other human nail and the author has seen several cases in which it was entirely missing. Furthermore, the terminal and middle phalanges of the little toe fuse, sometimes as early as fetal life, in at least three out of every ten whites and in eight out of every ten Japanese.[8] This high frequency in

[8] According to B. Adachi (Die Fussknochen der Japaner. Mitt. med. Fak. Univ. Tokyo, Vol. 6, 1905) and K. Hasebe (Ueber die Häufigkeit der Coalescenzen, Synostosen und Assimilationen der Fussknochen der Japaner. Zeitschr. Morphol. u. Anthrop., Vol. 14, 1912). The same fusion occurs also—though much less frequently—in Hottentots, Fuegians and Egyptian mummies.

the Japanese has rendered the variation of only two free phalanges the normal typical condition of that race, while in whites evolution has not gone quite so far. These examples show very clearly how phylogenetic changes are taking place to-day in the form of individual variations.

One of the most variable structures in man is the outer ear. Not only does it show very marked asymmetries, but it also fluctuates in size to a surprising degree, even in individuals of the same race and sex. Figure 11 shows

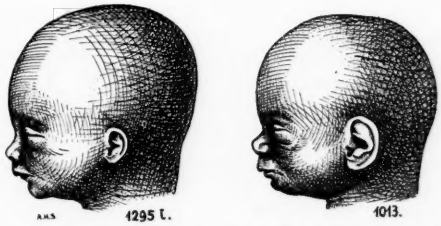


FIGURE 11. Minimum and maximum relative ear size in white fetuses of twenty weeks.

this wide range of variation in the relative size of the ear during fetal life. Its shape varies even more extensively; the detailed configuration of helix and anthelix is hardly ever the same in any two individuals. Upon finding such a high variability in the human ear we are justified in suspecting that this structure is undergoing some evolutionary change, in the form of either an increase or a decrease in size. There are no direct means of finding out whether the ears of our ancestors were larger or smaller. Comparative anatomy teaches only that the ear of man is of about the same size as that of the gorilla, but much smaller than that of the chimpanzee, and relatively larger than the orang ear, the last being the smallest of any primate ear.[9] Here embryology is in a position to cast a decisive vote. In chimpanzee, man and orang the ear in-

[9] Further data on this point can be found in the author's paper, "Embryological Evidence of the Evolution of Man" (J. Washington Acad. Sciences, Vol. 15, 1925).

creases in relation to the head size throughout fetal growth. After birth this increase continues steadily in chimpanzee, producing the enormous ear characteristic for this ape, whereas in orang and in man it reverses its trend to a decided postnatal relative decrease which, however, is not quite as great in man as in orang. This analogous ontogenetic process in human and orang ears makes it highly probable that the ears of man have been or are being reduced in size, similar to what happened in orang. This statement does not apply to the ear lobule, which is much more strongly developed in man than in the other primates, among which a cartilage-free lobule occurs really only in chimpanzee in the form of an exceptional variation. The human lobule is a progressive structure and as such varies also a great deal.

As a final example of the direct relation between variations and evolutionary modifications some conditions in the last molar tooth may be briefly discussed.[10] Any bulldog skull, with its short snout region, shows strikingly the effect of a shortened palate on dentition. In this race of dogs, not only are the premolars crowded and twisted, but the last molar is either rudimentary or, in most cases, entirely lacking, no bone being left for its support. A similar shortening of the palate has occurred in the evolution of man, particularly the orthognathous races, in which there exists an identical tendency for elimination of the most posterior tooth. This last molar is by far the most variable tooth in man to-day, certainly in the white race. In size, in the number of cusps and roots, and in the time of their eruption, the wisdom teeth fluctuate tremendously. These teeth have been reported to be missing entirely in as many as one out of every five whites. The third molar in man seems, therefore, to be doomed by evolution. It is interesting to find that the same process has, independently, of course, already been completed

[10] These conditions have been published in detail in another paper by the author: "Studies on the Evolution of Human Teeth" (*Dental Cosmos*, Vol. 67, 1925).

in other primates, and that in still others it is under way. All these forms are characterized by proportionately short palates. In the South-American marmosets the third molars have completely disappeared, while in the Spider monkeys these teeth vary almost as much as in man and are lacking in 15 per cent. of the cases. The normal hereditary endowment of man, and of the Spider monkey, calls for the development of three functioning molar teeth in each half of each jaw at a certain stage in ontogeny. This is the typical capacity for growth, but every now and then an individual will not reach this particular growth limit, possible for its species. In some the last molar will be merely belated, erupting in man as late as the fortieth year of life; in others the tooth will be represented by a vestige only, while in the most extreme cases the tooth is never laid down. As these individual variations may gradually become more and more frequent through being inherited, they will ultimately lower the average capacity for tooth growth in the type of the race or species.

In conclusion it should be emphasized once more that in this discussion of variations the author has quoted observations and figures which, he feels confident in stating, represent facts; they are all visible and measurable stages in nature's experiments on life. But in trying to interpret these findings, particularly in the attempt to throw light in the meaning of variations, it was necessary to enter the domain of hypotheses. New facts and different minds may alter these interpretations.

The data discussed seem to the author to support the idea that variability is primarily an independent and fundamental quality of life which is directly connected with, if not responsible for, evolution. Evolution will continue as long as variations occur.

THE RELATION OF THE SCROTUM TO GERM CELL DIFFERENTIATION IN GONAD GRAFTS IN THE GUINEA PIG[1]

DR. CARL R. MOORE

HULL ZOOLOGICAL LABORATORY, THE UNIVERSITY OF CHICAGO

INVESTIGATIONS into the biology of the testis conducted in this laboratory have given us certain new facts relative to some of the conditions that influence the production of spermatozoa, and as applied to the tissue reactions of transplanted testes, a procedure has been indicated whereby we have been able to so transplant testes from one animal into another as to obtain spermatozoon differentiation in the graft (for review of literature on the testis see Moore '26a). The writer has recently reported details on the differentiation of rat testis grafts and the factors influencing persistence and differentiation (Moore '26b). Briefly, it was found that not only do transplanted rat testes persist for months in normal and castrated males and in normal virgin, spayed or pregnant females, but that the range of variable differentiation in the grafts was essentially the same for all the different groups of animals, provided the grafts were localized in equivalent environments; by equivalent environments is meant positions within the animal body where essentially the same body temperature exists. We have determined that the normal environment of the testis (the scrotum) has an appreciably lower temperature than the abdomen and that such high temperatures as exist in the latter location prevent a testis from completing its spermatogenetic cycle (Moore '24 a, b, Moore and Oslund '24, Moore and Quick '24). By transplanting testes into the scrotum it was found, in the rat, that the

[1] This investigation has been aided by a grant from the Committee on Sex Research of the National Research Council; grant administered by F. R. Lillie.

spermatogenetic cycle could be completed in the transplanted testicle, and quantities of differentiated spermatozoa have been found in such grafts many months after transplantation (Moore '26b).

Since it has been pointed out before that rat testes were more easily transplanted successfully from one animal to another than were guinea pig testes, it appeared advisable both to determine if similar differentiation could be obtained in guinea pig testes transplanted into the scrotum of another guinea pig, and to report briefly the more limited success, but the actual attainment of such differentiation in transplants in this animal.

The effectiveness of differential temperatures having proven so important in the behavior of testes and testis grafts, we desired also to learn whether the ovary might likewise be affected if placed in an environment different from that to which it is normally subjected. This latter inquiry has so far limited itself to a study of ovary grafts from different regions of the animal body and in particular to grafts that have been made in the scrotum.

A brief report of the results from transplanting testes and ovaries into the scrotum of the guinea pig follows.

Materials and Methods

The guinea pigs used in these experiments are the products of our own large colony. Despite the introduction of outside males, approximately each year, the colony is obviously somewhat inbred; numbering some hundreds of animals, the recipient and donor of the graft have the chance of a close relationship, but in all cases the donor has been considerably younger than the host. No particular attention has been given to selection in regard to nearness of relationship.

I have earlier reported testis and ovary transplantation in the guinea pig wherein the grafts were subcutaneous or intraperitoneal in position (Moore '21). The present report, therefore, will be restricted to grafts that have been recovered from the scrotum some months after transplantation.

In making such transplantations the abdomen of the male host, thirty to one hundred days old, is opened under either anesthesia and the testes retracted into the abdomen through the open inguinal canals, bringing with each the everted lining of the scrotal pouch (tunica vaginalis). Removal of the entire testis and epididymis is followed by sewing, with fine sterile white silk, an entire testis with its attached epididymis (or an ovary) from an animal of three to fifteen days of age killed immediately before the testes of the recipient animal are removed. The graft is sewed to one wall of the tunica vaginalis, scarified with a needle point or small scalpel, and the scrotal pouch turned back into its normal position. By this procedure the graft becomes located inside the scrotal pouch in the normal position of the testis without causing the injury to the scrotal skin by an incision. In sewing the testis, or ovary, in place the silk ligature passes directly through the organ and the suture so tied as to hold it in direct contact with the scarified area; thus an entrance is provided for ingrowing blood vessels, and in the case of the transplanted testicle several needle punctures of the tunica albuginea have often been made to facilitate vascularization.

In such a method of transplantation, a testis, with its attached epididymis, is kept intact by its own confining boundaries and the seminiferous tubules are not so widely spread apart as they are when cut pieces are subjected to the ingrowth of considerable amounts of host connective tissue. It is realized that the organ is less easily vascularized than a cut piece, but better results have been obtained using the entire young testis than a portion of an older one. The added feature of transplanting a testis long before spermatogenesis has been established (in guinea pig about forty-five to sixty days) is important in deciding definitely the origin of spermatozoa that may be present in the graft some months later; when immature testes are transplanted it becomes obvious that spermatozoa found later must have been dif-

ferentiated subsequent to the establishment of vascularity and renewed activity in the transplant.

Results

Testis transplantation: Despite the fact that several additional guinea pig testis grafts from subcutaneous locations have been studied since my earlier report (Moore '21) no new facts of a fundamental importance have been obtained and I shall, therefore, present here only the new facts concerning differentiation in scrotal transplants.

It has been emphasized earlier that the scrotum is a very poor location for obtaining successful "takes" of testis transplantations due to the relatively poor vascularity of the part. Furthermore, I have pointed out, for the rat (Moore '26b), that all persisting testis grafts do not consist of seminiferous tubules with an active germinal epithelium. The graft, to carry on spermatogenesis, must have established a vascular connection before complete necrosis, and must have retained cells within the tubules capable of generating the seminal line. Transplanted testes in other locations, though often more favored for the acquisition of vascularity, have similar obstacles to overcome, but may persist for months with the seminiferous tubules lined with an active germinal epithelium and yet not produce spermatozoa; despite the active spermatogenesis with many tubules showing quantities of dividing spermatogonia and spermatocytes, and some with questionable spermatids amid the degenerating cells, spermatozoa have never been seen in subcutaneous or intraperitoneal guinea pig testis grafts. The cells of the germinal line differentiate, therefore, to the spermatocyte stage, but instead of completing the cycle the cells escape from the epithelium into the lumen and degenerate.

Among the scrotal grafts, as in similarly located grafts in the rat, all grades of graft persistence and reaction have been obtained. Some have been so completely absorbed that only epididymis tubules have been retained,

or complete absorption may have taken place; others show seminiferous tubules well outlined but wholly inactive in spermatogenesis; still others may show a few active tubules, but with the majority of the tissue necrotic or totally resorbed and replaced by connective tissue.

The amount of seminiferous tubule mass that has resisted the forces antagonistic to its retention seems not to influence the grade of differentiation as will be seen from a detailed consideration of the following case:

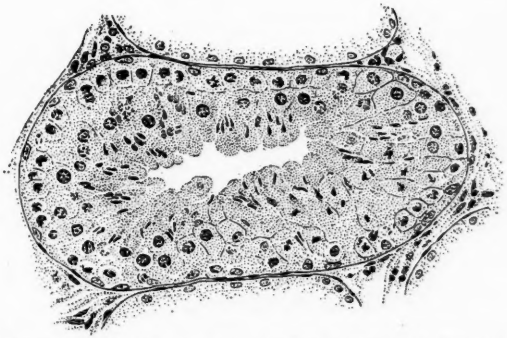
Dec. 6, 1923, a 60-day-old male guinea pig (107A–B–181) from stock; left testis removed and whole testis from 14-day male sewed to tunica vaginalis; second testis transplanted subcutaneously in neck. Right testis of recipient undisturbed. May 21, 1924, animal killed. Both scrotal and subcutaneous grafts persist (5½ months) well vascularized. Right testis present and normal.

In this case the two testis grafts had persisted for five and one half months along with one testis of the host animal. The subcutaneous graft, on histological section (serially), proved to have been very poorly preserved. Many well-rounded seminiferous tubules were present, but there was no spermatogenetic activity. There was considerable fibrosis and leucocytic infiltration.

The scrotal graft was found, after serial section, to consist, for the most part, of epididymis; very rough estimates indicate the gross amount of seminiferous tubule mass in comparison with epididymis, as approximately 1 to 10. This small amount of the testis portion proper consists of seminiferous tubules in all stages of differentiation from entirely quiescent tubules, with little or no cells and no mitoses, through tubules with an active germinal epithelium to tubules that contained differentiating spermatids and many spermatozoa. The majority of the relatively few tubules present contain an active germinal epithelium. In several of these one can see loosened cells in the lumen undergoing degeneration, but in other tubules the normal structure has been retained. Grades of the spermatogenetic cycle exist here as in the normal testes; one tubule may contain largely spermatids with division figures in spermatogonia; other tubules show large numbers of spermatocytes or mainly metamorphos-

ing spermatids with sperm heads beginning to be apparent. Still other tubules contain many spermatozoa. Fig. 1 shows a cross section of one tubule containing well-differentiated spermatozoa.

The intertubular tissue is essentially normal in amount in this graft.



Seminiferous tubule from guinea pig testis graft (107B–181). Testis transplanted to scrotum, from an animal fourteen days after birth, and removed five and one half months later. Spermatozoa present, mitoses numerous. Drawn by Kenji Toda.

Here, therefore, is a graft of a guinea pig testis transplanted long before spermatogenesis had begun, into the scrotum of a considerably older animal, one of whose testes had remained undisturbed and normal; after five and one half months in the scrotum, and after the major part of the transplant had undergone necrosis and resorption, a few tubules have been vascularized sufficiently early to permit of their retention and differentiation. The majority of the tubules remaining have continued to carry on spermatogenesis, all elements of which can be seen, and several of these had been, and were, differentiating spermatozoa at the time of graft removal. Among mammals the only other testis grafts reported to produce spermatozoa were from the rat (Moore '26b) with the questionable inclusion of the ram and goat (Voronoff, Retterer; see discussion Moore '26b). This affords further evidence for our hypothesis that testes must reside

in, or sufficiently near to derive the influence from, the scrotum. I have recently reported for the guinea pig that the animal's own testis, situated subcutaneously outside the scrotum, and having suffered no interruption of its own blood supply, ductus deferens, or nerve connection, continues its spermatogenetic function for months, but has so far in our experience been unable to produce spermatozoa (Moore '26c). This is due to the loss of cells from the epithelium lining the tubules and their consequent degeneration. Additive evidence that the scrotum regulates the environmental temperature, under which regulation spermatogenesis is normal, serves to emphasize the gradations obtainable in testis activity from the abdominal cavity outward. In the abdomen, activity of the germ cells is in abeyance; artificial retention of a normal testis in the abdomen is rapidly fatal to the germinal cell line of the testis (Moore '24a). Underneath the unmodified skin the animal's own testis with all its proper connections, or a testis graft, continues in activity up to about the spermatocyte stage; but a testis or a testis graft in the scrotum can succeed in producing spermatozoa. Since the transplantations here discussed were made before the onset of spermatogenesis, there is no question that the spermatozoa have been, and were being, produced within the graft itself at the time of removal and are not merely spermatozoa retained from differentiation before transplantation. This gradation of activity in the testis from within the abdomen outward is associated with the gradations in temperature and, with our collective evidence reported previously, there is little question but that the temperature factor is the primary cause of the differences.

The temperature within the guinea pig scrotum alongside the testis in its normal position, as measured with an ordinary 100° C thermometer at a normal room temperature, is approximately 2° C lower than that of the abdomen (Moore and Quick '24); the difference is even greater when records are taken in a room temperature below normal. Though it may appear incredible that

such a slight difference in temperature could be so important in testis activity, one has but to confine the testis of a normal breeding male in the abdomen for ten days to see the complete disorganization of the seminiferous tubule epithelium. Under such a disorganized, degenerate condition the testis will completely recover and resume the production of normal germ cells if it is returned to the scrotum, whereas, if left in the abdomen it not only never again resumes the spermatogenetic activity, but actually becomes more degenerate. At the end of one year's retention in the abdomen the testis is an extremely minute structure, wholly quiescent in spermatogenesis. If incomplete descent follows elevation then an incomplete spermatogenetic function follows. Again, if one passes a continuous stream of water, warmed to 10°C above its normal body temperature, for thirty minutes (one application only) over the scrotum of the guinea pig the testis, within ten days, will show a high grade of degeneration progressive through the tissue from the point most exposed to the warmed water to that of the deeper tissues less subjected to the higher temperatures. The animal will sterilize itself with its own normal body heat within ten days (guinea pig) if the testis is confined continuously in the abdomen; or again it will be sterilized, temporarily at least, by one, thirty minute exposure of the scrotum to water raised 10° C above the normal body temperature.

It is most certainly the temperature gradation that explains these, and the many other phenomena we have described, including the differentiation existing as between subcutaneous grafts (or the animal's own testes) and scrotal grafts. Scrotal testis grafts, if incorporation in the animal body has been sufficiently successful, will produce spermatozoa, whereas subcutaneous grafts have as yet failed to do so. Among the hundreds of testis grafts studied personally not one has produced spermatozoa except those residing in the scrotum.

Ovarian grafts: I have previously discussed the structural condition and effectiveness of ovarian grafting in

both the rat and the guinea pig (Moore '19, '21) and wish here to add but few words in reference to this subject. My chief interest in this additional study of ovarian grafts was to determine whether the scrotal environment would in any way affect the differentiation of the graft, since the latter organ is normally in a position where it is subjected continuously to higher temperatures than the testis will tolerate and carry on its normal function.

Ovarian grafts residing in the scrotum of males totally castrated or semi-castrated and grafts adherent directly to the testis itself for months, have constituted the new materials. Such grafts, however, have failed to indicate any deleterious influences from the lower temperatures of the male scrotum. These, as in earlier reported subcutaneous and intraperitoneal grafts, consist of the characteristic ovarian stroma with normal Graafian follicles containing ovocytes in all stages of development from primordial follicles to more nearly mature ones. The earlier work of Sand ('22, '23) and of Lipschutz ('24) has shown that an ovarian graft imbedded within the substance of a testis resident (at least sometimes) in the normal scrotal position, will continue its follicular growth and differentiation. Confirming this I can add that in the empty scrotum of a castrated male the ovarian graft will continue active for some months without revealing any ill effects attributable to the strange environment.

SUMMARY AND CONCLUSIONS

(1) The testis of young guinea pigs can be transplanted onto the walls of the scrotal sac, thus being brought into the normal environment of the testis, and persist for months.

(2) Such scrotal grafts, even after the majority of the graft has undergone necrosis and removal, may consist of seminiferous tubules with all grades of the spermatogenetic cycle represented, including quantities of differentiated spermatozoa (five and one half months after transplantation).

(3) All grafts resident in the scrotum are not sufficiently well incorporated within the animal body to make possible the production of spermatozoa.

(4) The cause underlying the production of spermatozoa in mammal testis grafts in the scrotum, and the lack of such development of subcutaneous grafts is the heat regulatory function of the scrotum.

(5) Ovarian grafts in the scrotum of rats or guinea pigs fail to show any effects attributable to the lower scrotal temperature and hence are believed to be less sensitive to changes in temperature than are the testes.

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HETEROPLOIDY AND SOMATIC VARIATION IN THE DUTCH FLOWERING BULBS[1]

WILLEM EDUARD DE MOL

It is not my purpose to give at this time a complete description of the somatic variations in hyacinths, tulips and narcissi, which I have been studying during the past seventeen years. I shall try to give only a short review, in which the following questions are principally considered. (1) Are there in heteroploid varieties more somatic variations than in diploid varieties? (2) Is the somatic variation in color or form attended by changes in chromosome numbers?

Somatic variation of the flower coloration may occur without change in chromosome composition. The triploid variety of hyacinths called Grand Maître has a light bluish violet color. The following bud variations have been observed in this variety: (1) light salmon; (2) very light carmine with light violet; (3) blue violet; (4) light violet; (5) cobalt blue. The bud variations 1 to 3 show a great deal of stability in color during vegetative propagation, while 4 and 5 return very readily to the original type. In hyacinths the anthocyanin occurs as a rule in the cells of the subepidermis. In very dark-colored varieties the anthocyanin is found in the epidermis as well as in the subepidermis. In the case of Grand Maître and its bud variations it occurs, with one exception, exclusively in the subepidermis. The exception is that of the bud variation that is very light carmine with light violet, in which case the anthocyanin of the well-opened flower is found only in the epidermis. My study concerning changes of flower coloration by somatic variation has included varieties which have existed for more than a cen-

[1] Report given before the American Society of Naturalists and the Joint Genetics Sections at Yale University, New Haven, Connecticut, U. S. A., December the 29th, 1925.

tury, and it has been possible to compare the changes in flower coloration of diploid and heteroploid varieties. This has led—at least previsionally—to the conclusion that the multiplicity of somatic variation in flower coloration has not at all or very little increased in these cultivated varieties whose somatic number of chromosomes differs from the diploid number.

I have also been able to collect in my own cultures and from the growers rather complete evidence as to somatic variation in the color of tulips. Surprisingly large is the constant repetition of the number of changes in color which may occur because the anthocyanin, as a result of somatic variation, changes to a lighter or a darker shade or is developed in larger or smaller quantities. If there is added to this the somatic variation caused by change in the color of the carotinoids in the chromoplasts and the somatic variation caused by both these phenomena, then it will be clear that it has been possible to describe in the case of the well-known single early tulip La Reine more than fifty bud variations and in the case of the equally well-known double early tulip Murillo more than forty. To the most peculiar variations belong those in which a change of color occurs at the same time with changes of form and dimensions. It has never been shown that a certain change in color occurs at the same time as a change in form. It is remarkable how many times the combination of vermilion red with chrome yellow has turned up.

An important point is the so-called "breaking of the flower coloration" of tulips, *i. e.*, the appearance of yellow and white blotches and stripes as a result of the failure of the anthocyanin to develop or only to develop slightly in particular regions. It is impossible to go further into this matter at the present time, but in my opinion this phenomenon does not occur outside the limits of the other somatic variations. I consider it to some extent due to the perseverance of the pattern at the base of the tepals, to which in other cases also the somatic variation must often be attributed.

In conclusion, then, it may be said that in tulips as well as in hyacinths the somatic variation of diploid varieties is not less conspicuous than in the case of varieties which have another number of chromosomes in the somatic nuclei.

In narcissi, anthocyanin and other pigments which are dissolved in the cell sap are absent. The color is caused by chromoplasts which can be recognized during the flowering period either as more or less globose, yellow bodies (*e. g.*, in Narcissus Pseudonarcissus) or as crystals, varying in color from orange to brown (*e. g.*, in the border of the corona of Narcissus poeticus). I have found also in this case that the color can brighten or deepen as a result of somatic variation. However, no direct relation between heteroploidy and the number of somatic variations has as yet been proved.

Changes in form due to somatic variation may also occur without any change in chromosome composition. For example, the triploid hyacinth Grand Maître has given rise by somatic variation (a) to forms in which all organs, except probably the roots, have greatly increased in length; (b) to forms in which all the tepals are provided at the top with a peculiar shaped threadlike appendage. Both somatic variations maintain themselves constantly.

The parrot forms in tulips originated as a result of somatic variation from varieties with normally shaped flowers. It is to be noted that the parrot type is not always as sharply defined as might appear to be the case when commercial varieties are observed. There are a great many intermediate forms between the normal flower and the parrot type which remain constant during vegetative propagation. Also those flowers should be considered in which a green color is very prominent and in which all organs or at least the tepals have elongated exceedingly and in this respect and also often in their spiral arrangement the floral parts resemble the leaves in character.

Splitting of the corona in cultivated narcissi may occur either opposite the middle of the tepals, or back of the openings between the tepals. These changes which have originated as somatic variation have been described in volume I of my book: "De wetenschappelijke beteekenis van de veredeling der Hollandsche bloembolgewassen"—"The Scientific Significance of the Improvement of Dutch Flowering Bulbs." These and other somatic variations have led to some new viewpoints concerning the morphological meaning of the paracorolla of the Amaryllidaceae in general and of narcissi in particular.

While in hyacinths and in narcissi most bud variations involving color and form are to be found in heteroploid varieties, this should not be attributed, I think, to the fact that heteroploidy more than diploidy appears to be connected with the origin of such changes, but rather to the fact that diploid hyacinths and narcissi—more especially varieties of Narcissus Pseudonarcissus and hybrids between this species and another—have become rarities in comparison with the number of grown heteroploid forms.

In tulips, on the contrary, diploid varieties constitute by far the larger part of the grown varieties. This is the only reason that in this species somatic variation is found most often in diploid forms.

The cases of somatic variations which are caused wholly or partly by a decrease or increase of the number of chromosomes are rare. Modifications in size or form of the individual chromosomes have never been observed.

In tulips it has not been possible to prove that bud variations are related to the occurrence of new chromosome numbers. Perhaps this can be attributed to the fact mentioned above that, in general, in Tulipa Gesneriana diploidy has persisted to a greater extent than in Hyacinthus orientalis and Narcissus Pseudonarcissus.

In hyacinths it has been shown that in rare cases bud variations arise whose somatic nuclei contain a different

number of chromosomes from that of the mother variety. The so-called "dwarf types" belong here. These somatic variations originated from the triploid variety King of the Blues (24 chromosomes) and are characterized respectively by eighteen and twenty-one chromosomes in the somatic nuclei. Besides their dwarf-like habit they are distinguished principally from King of the Blues by the color of the flower, which has changed from indigo into red violet. It is difficult, however, to determine in how far the modification of chromosome number is the cause of the change in color since King of the Blues has also given rise repeatedly to somatic variations known as Queen of the Pinks, in which the triploid number of chromosomes has been maintained. The color of the flower of the latter is light violet with carmine, which is not very different from that of the dwarfs. The dwarf type with twenty-one chromosomes has appeared three times to our knowledge, probably because special attention was paid to somatic variation in King of the Blues as soon as the first dwarf had been found. Afterwards other parallel cases were ascertained regarding other heteroploid varieties. In Narcissi tetraploid somatic variations of diploid hybrids between Narcissus Pseudonarcissus and Narcissus poeticus have been discovered.

Thus among the hundreds of bud variations of the principal varieties of flowering bulbs there are only a few rare cases of concomitant changes of chromosome number or of nuclear structure. This fact shows that heteroploidy having manifested itself so strongly in hyacinths and narcissi and being observed also in tulip cultures should not be connected in the first place with the process of somatic variation. In the above-mentioned cases it is not the sporophyte which changes easily its nuclear structure, *i. e.*, its number of chromosomes by external or internal influences. It is rather the gametophyte.

As far as heteroploidy of Dutch varieties of hyacinths is concerned the cause or at least one of the causes of it has been found. Since 1921 it has been evident that pollen

grains with an increased number of nuclei or with diploid nuclei can be produced at will and in 1922 the first triploid descendants were obtained by the union of a haploid egg and a diploid pollen grain.

Summary

The following is a summary of somatic variations in hyacinths, tulips and narcissi.

A. Somatic variation without change of chromosome number.

(1) Somatic variation of flower coloration.

In hyacinths: heteroploid varieties without change of chromosome number produce bud variations in which the anthocyanin appears in the epidermis instead of in the subepidermis. The blue color changes into light violet.

In tulips: carotinoids as well as anthocyanin act a part in somatic variation. Moreover, sectors are found in which form and color are different from the mother variety.

In narcissi: changes of the color of the chromoplasts take place by somatic variation.

(2) Somatic variation of form.

In hyacinths: somatic variations of triploid varieties occur in which one organ or all the organs of the plant are much elongated.

In tulips: besides parrot forms very primitive shaped flowers appear.

In narcissi: splitting of the corona by somatic variation and phenomena connected with it occur.

B. Somatic variation with change of the chromosome number.

In hyacinths: hypotriploid forms arise from triploid varieties characterized by eighteen and twenty-one chromosomes in the vegetative nuclei. These somatic variations change the flower color from blue into red.

In narcissi: tetraploid forms originate from diploid hybrids between Narcissus Pseudonarcissus and Narcissus poeticus.

EIGHT GENERATIONS OF SELECTION WITHIN A CLONE OF HELMINTHOSPORIUM SATIVUM[1]

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INTRODUCTION

STEVENS (1) in working with *Helminthosporium sativum* found that the parasite, while being cultured in the laboratory, was very susceptible to even slight changes in the environment. These changes were expressed as variations in growth rate, color, aerial mycelium, shape, septation, size of the conidia, etc.

RAVN (2) had previously mentioned that septation in this organism was quite variable, so much so that it amounted to specific differences. Since there are only three common descriptive characters in this genus, shape, size and septation of the conidia, it is apparent that any one of them assumes considerable importance in identifying the species.

However that may be, the fact that the organism is so variable makes it a desirable one with which to carry on selection problems. With this thought in mind it was planned to make selections within a clone and attempt to change the mean number of the septa.

SOURCE OF THE ORGANISM

A transfer was made of *Helminthosporium sativum* from a stock culture in the laboratory. The culture was

[1] Teachers of genetics will no doubt be glad to have their attention called to a short-eyeled species of plant suitable for demonstrating in the laboratory certain of the principles of their subject. The accompanying brief papers of Messrs. Miller and Ayers are two of the term reports on laboratory work that was part of a semester's course on selection. The matter presented represents also a contribution, though small, to the literature of selection.—J. P. Kelly, Department of Botany, Penn. State College.

Contribution of the department, number 48.

from an isolation originally made by Mr. W. A. Kuntz, from infected roots of wheat seedlings, at Madison, Wisconsin, in 1922. The pathogenicity of the organism was later proved in work conducted in the laboratories at that place.

Procedure

Facilities made it impossible to carry on this work under thoroughly controlled conditions, but as regards the important factor of the medium a more or less constant condition was secured.

Several methods were employed in attempts to isolate single spores before a good one was found. The method which was the most usable was to lightly touch a sterile needle to the fruiting surface of the colony and transfer the mass of spores to a small quantity of sterile water in a test tube. After agitating the tube by shaking, three or four loopsfull were transferred to a tube of melted agar, which was shaken and the contents poured into a sterile petri plate.

In this manner a satisfactory dilution of spores was obtained. The inoculated plates were allowed to stand at laboratory temperature until growth of the mycelium was visible to the naked eye. At this time a dozen or more patches of growth would show up on the agar, which could readily be examined under the microscope and the number of cells of the spores determined. When a spore of the desired number of cells for a particular culture was found it was cut out along with a portion of the agar and the whole transferred to a slanted tube of agar. The organism was permitted to grow here until the surface was blackened with the matured conidia, at which time the counts were made.

In preparing material for counting, a small quantity of the fruiting surface was removed with a sterile needle and mounted under a rectangular cover slip. Determinations were begun at one end and continued by moving the slide in only one direction. When the other end was

reached a new mount was made. In this manner most of the danger of counting the same spore twice was removed.

RESULTS

The clone was begun with a seven-celled spore taken from the original isolation. From the progeny of this parent spore the development of a plus strain was attempted by starting with a ten-celled spore and repeatedly choosing for subsequent generations spores with high cell numbers. Similarly, a minus strain was attempted, beginning with a five-celled spore and carrying it on through few-celled spores. All the material presented, then, is descended from the one seven-celled spore originally separated. After eight generations the mean for each strain was determined. The parental spores and the means for each generation are given in Table No. I and Chart I.

TABLE I

EIGHT GENERATIONS OF SELECTION IN CLONE OF HELMINTHOSPORIUM SATIVUM

Generation	"Strain"	No. of Spores	Frequency 3	4	5	6	7	8	9	10	11	12	Mean	Spore Parent
1		200	1	3	28	42	33	45	28	14	4	2	7.34 ± .0821	7 Celled
2	Plus	"	1	6	19	32	40	49	29	16	6	2	7.985 ± .088	10 "
	Minus	"	1	1	9	17	26	49	47	30	15	5	8.38 ± .084	5 "
3	Plus	"	1	1	7	28	46	57	42	14	3	1	7.75 ± .083	9 "
	Minus	"	1	3	9	33	61	50	27	8	7	1	7.45 ± .0762	3 "
4	Plus	"	1	4	6	25	50	34	60	10	6	4	7.08 ± .0956	9 "
	Minus	"	1	4	27	40	35	45	25	17	4	2	7.31 ± .0847	4 "
5	Plus	"	2	2	25	40	45	30	45	6	4	1	7.22 ± .088	11 "
	Minus	"	1	2	40	45	38	50	17	4	2	1	6.82 ± .0861	5 "
6	Plus	"	1	5	15	45	41	30	35	20	5	3	7.45 ± .088	9 "
	Minus	"	5	8	30	25	37	49	28	14	3	1	7.17 ± .0856	3 "
7	Plus	"	1	3	20	35	42	51	32	10	2	4	7.42 ± .0827	8 "
	Minus	"	1	3	25	38	40	47	30	12	3	1	7.29 ± .0784	4 "
8	Plus	"	2	5	15	24	31	48	50	15	5	5	7.8 ± .099	9 "
	Minus	"	1	5	31	33	47	48	29	3	2	1	7.7 ± .0741	4 "

It is seen that while the sizes of the parental spores for each generation differed widely, the averages of the progenies (plus and minus) were nearly the same, so that the attempt to separate the single clone into two by selection proved ineffective.

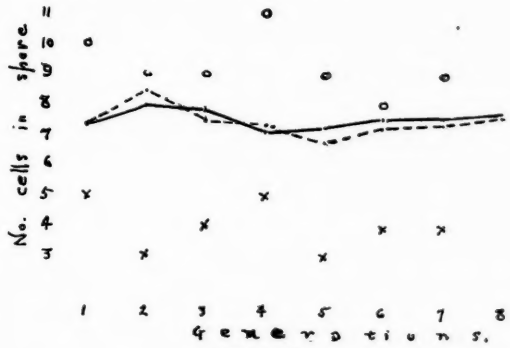

CHART I

All individuals entering this chart descended from *one* 7-celled spore.
———— Course of means of attempted plus strain.
- - - - - - - - Course of means of attempted minus strain.
O marks position of plus spore selected to give succeeding generation.
X means position of minus spore that was to give next generation.

A review of the literature shows the consensus of opinion to be that selection within a clone or pure line is not effective in changing the character of the progeny. The results from selection within a clone of *Helminthosporium sativum,* as given in this report, are in harmony with this idea. It will be noted from the graph comparing the means of the eight generations that while there are slight deviations, they are not permanent, and that at the end of the eighth generation the averages of the two attempted strains are practically at the same point. Selection has not produced two strains.

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SELECTION WITHIN A CLONE OF HELMINTHOSPORIUM SATIVUM DURING SEVEN GENERATIONS

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Helminthosporium sativum belongs to that group of fungi known as the Fungi Imperfecti. The fungus possesses branched and septate hyphae. The conidia are borne solitarily and are dark brown in color, being 4–12 septate; the size of the conidia ranges from 105–130 × 15–20 μ; the shape of the conidia is elliptical. The fungus was grown on corn meal agar throughout the experiment,

TABLE I

A SURVEY OF THE EFFECT OF SELECTION WITHIN PURE LINES

Generation	Strain	Frequency 3	4	5	6	7	8	9	10	11	12	13	Total spores	Mean	Duration of generations	Remarks
C_1		1	6	15	27	45	59	65	31	15	2		266	7.94 ± .0701	12 days	Parent was a single 7-celled spore
C_2	C_2+			6	19	36	54	64	31	8	6	1	225	8.40 ± .068	12 days	Parent 9-celled spore
	C_2-	1	3	7	32	50	57	40	19	5	1		215	7.78 ± .068	10 days	Parent 5-celled spore
C_3	C_3+	1	6	26	52	66	47	23	9	1			231	6.76 ± .044	12 days	Parent 9-celled spore
	C_3-	1	10	24	36	57	46	26	17	3	3		223	7.18 ± .077	13 days	Parent 5-celled spore Agar dried out
C_4	C_4+		5	25	41	65	39	22	11	2			210	6.85 ± .069	13 days	Parent 9-celled spore
	C_4-		7	21	46	72	33	13	9	3	1		205	7.01 ± .067	9 days	Parent 5-celled spore
C_5	C_5+		2	9	19	46	56	42	17	8	3		202	7.92 ± .071	10 days	Parent 9-celled spore
	C_5-		1	7	23	47	63	45	29	3	2		220	7.78 ± .063	10 days	Parent 5-celled spore
C_6	C_6+			14	30	63	59	24	23	5			218	8.00 ± .064	24 days	Parent 9-celled spore
	C_6-		14	49	58	46	25	6	3				201	6.23 ± .061	24 days	Parent 5-celled spore
C_7	C_7+		3	28	40	72	37	14	1	1			200	7.13 ± .043	11 days	Parent 9-celled spore
	C_7-		5	28	49	78	35	16	4	1	1		216	6.82 ± .058	11 days	Parent 9-celled spore

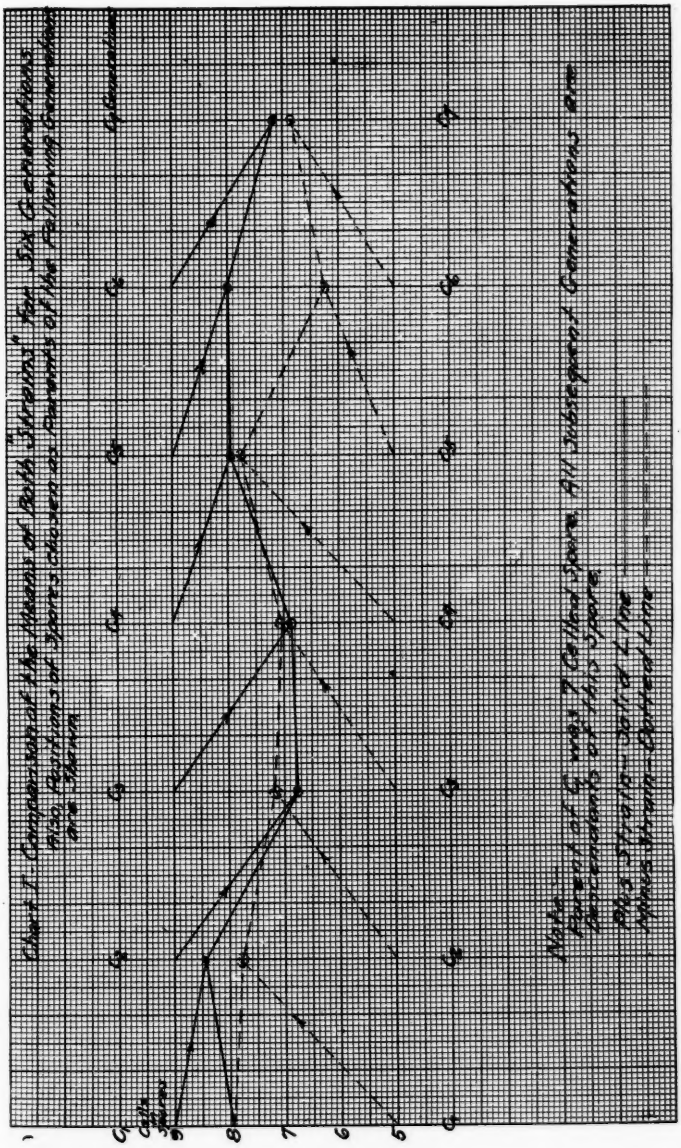

CHART I

one batch of the agar being prepared at the beginning and this lot lasted the entire experiment (2).

The work was started from a single seven-celled spore which germinated, grew and fruited, giving the generation marked C_1 in Table I and Chart I. Selection of a nine-celled spore and of a five-celled spore was made from generation C_1 in the attempt to develop sub-strains containing conidia or spores with high number of cells and low number of cells. Generation C_2 gives the first opportunity to see if our selection is effective. In C_2 the descendants from the 9-celled spore averaged 8.40 cells per spore and the descendants from the 5-celled spore averaged 7.78 cells per spore. (See Table I and Chart I.) To get the next generation (C_3) a nine-celled spore was isolated from the plus "sub-strain" of generation C_2 and a five-celled spore from the minus "sub-strain." In C_3 their respective progeny averaged 6.76 cells and 7.18 cells per conidium or spore. The minus sub-strain averaged slightly higher than the plus sub-strain.

Similar selections were made in each generation and the results through the seventh generation are given in Table I and Chart I. The means of both sub-strains vary considerably sometimes in the direction of selection and sometimes not. As far as this experiment was pursued it indicates that selection within a clone of *Helminthosporium sativum* is non-effective. This agrees with C. D. LaRue's results with clones of Pestalozzia (1).

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(2) Stevens, F. L., "The Fungi which cause Plant Diseases." MacMillan Co., 1921.

CULTURE MEDIA FOR DROSOPHILA. I. CHANGES IN HYDROGEN ION CONCENTRATION OF THE MEDIUM[1]

PROFESSOR RAYMOND PEARL AND W. B. D. PENNIMAN

I

For a number of years past one of us (R. P.) has been studying the duration of life of the fruit-fly, *Drosophila melanogaster,* and the factors which influence it.[2] As this work has progressed it has become more and more evident that the degree of quantitative precision desirable in experimental actuarial work was not attainable when the flies were grown upon a medium which had as its chief ingredient so variable a product, seasonally and otherwise, as the banana. *Drosophila* is now widely used as a laboratory animal, especially in genetic investigations, following the lead of Morgan and his students. It is generally cultivated upon the following medium, which originated in Morgan's laboratory:

H_2O	500 c.c.
Agar-agar	10 gr.
Banana pulp	500 gr.

Boil agar until dissolved—about 10 minutes. Mash bananas and add to agar and water and boil for five minutes. Bananas must be ripe but not rotten. Pour into bottles for use. Allow to cool and sprinkle lightly with yeast.

Some slight variations in the preparation of this medium, such as neutralization to litmus before adding yeast, allowing the flies themselves to seed the medium with yeast, etc., have been practiced by various workers, but the essential character of the medium as cooked ba-

[1] From the Institute for Biological Research of the Johns Hopkins University. In the experiments here described the authors were assisted by Dr. Mary Gover.

[2] See a series of papers under the general title "Experimental Studies on the Duration of Life" in the American Naturalist, Vols. 55 to 58, 1921–24.

nana pulp solidified with agar has not been altered as the standard laboratory procedure in working with this fly. In their most recent publication on the biology of *Drosophila,* Morgan, Bridges and Sturtevant[3] have the following to say regarding food (*loc. cit.,* p. 6–7):

> For laboratory purposes, bananas furnish by far the most desirable culture medium, but the fly can also be reared on other fermenting fruits. In our culture bananas are used, but whether there is something supplied by the banana to the flies that is advantageous, or whether the banana is the best medium for the growth of yeast, that appears to be the chief element of the food for both flies and larvae, is not known. It is not improbable that the relative absence of moulds such as mucor, etc., in the acid banana cultures also plays a rôle in the results.

The work of Guyénot,[4] Loeb[5] and Northrop,[6] and Baumberger[7] has shown that any notion that fruit in any form is in any way necessary for any biological process in *Drosophila* is not true. Baumberger found that the flies "can develop normally on yeast nucleoprotein, sugars, and salts."

Our present interest in the matter is mainly practical; namely, to get an easily handled, standard food, which shall be of chemically constant composition, and therefore not a variable factor in biological experiments with the fly, and shall at the same time be not merely capable of sustaining life, but shall furnish something approaching an optimum environment from the standpoint of fly husbandry.

II

Early in the course of our experiments with fly foods it became desirable not only to determine the pH of the

[3] Morgan, T. H., Bridges, C. B., and Sturtevant, A. H., "Bibliographica Genetica," Vol. II, pp. 1–262, 1925.

[4] Guyénot, E., Comptes-rendus soc. biol. Paris. T. 74, pp. 97–99; 178–180; 223–225; 270–272; 332–334; 389–391; 443–445. 1913. *Bull. Biol. France et Belg.* 1917.

[5] Loeb, J., and Northrop, J. H., Proc. Nat. Acad. Sci. Vol. 2, p. 8, 1916. *Jour. Biol. Chem.,* Vol. 27, pp. 309–312, 1916. *Ibid.,* Vol. 32, 103–121, 1917.

[6] Northrop, J. H., *Jour. Biol. Chem.,* Vol. 32, pp. 123–126, 1917. *Ibid.* pp. 181–187, 1917.

[7] Baumberger, J. P., *Jour. Exp. Zool.,* Vol. 28, pp. 1–81, 1919.

media we were using, but also to find out how this characteristic changed with the life of the culture. It is the purpose of this paper to present some experimental results on these points.

The first series of experiments was of a preliminary character, and the results need not be given in detail here, because their only function was to aid in making the plans for the more extensive and thorough series discussed in the next section.

In summary the plan and the results of the first series were as follows: Two food culture media were compared, *viz.* (a) the standard banana medium described above, and (b) a synthetic medium prepared in the following way:

Stock Solution—S–99

Cane sugar	33.3 gm.
Dextrose	33.3 gm.
$KNaC_4H_4O_6 4H_2O$	6.7 gm.
$(NH_4)_2SO_4$	1.6 gm.
K_2HPO_4	.8 gm.
$MgSO_4 7H_2O$	.4 gm.
$CaCl_2$	.2 gm.
H_2O to make 400 c.c. of solution.	

Dissolve 4 grams of agar-agar in 100 c.c. of water, in autoclave. Bring solution to 100 c.c. by addition of water after agar is completely dissolved. Then add 100 c.c. of stock solution, thoroughly mix and pour in bottles. The solution sets to a moderately stiff jelly on cooling. When cool it is lightly seeded with powdered dry Magic yeast.

In the experiments approximately the same amount of medium, about 5 c.c., was poured into one-ounce straight-side vials in both the banana series and the synthetic

TABLE 1

CHANGES IN pH OF MEDIUM UNDER DIFFERENT CONDITIONS

No. of bottles	Conditions and time	Mean pH Banana	Mean pH S-99
3 + 3	Fresh medium—No yeast—No flies	4.9	7.1
3	Yeast 2 days—No flies	.....	4.9
6	Yeast 2 days—Flies 1 day	.....	4.0
6	Yeast 2 days—Flies 2 days	.....	3.5
6	Yeast sown with flies—Flies 2 days	3.45	.....

series. The bottles with the S–99 medium were incubated at 25° C. two days before the flies were added. Yeast was added to the banana bottles only at the time the flies themselves were put in. That is, there was no preliminary period of yeast incubation on the banana medium. Fifty flies were put in each bottle, and the bottles placed in a 25° electric incubator. At the end of one and of two days bottles were withdrawn and the pH of the medium determined by the colorimetric method.[8]

These preliminary results suggested the following relations:

(1) The fresh synthetic medium, S–99, before either yeast or flies are added to it, is much more alkaline in its reaction than is the banana medium under the same conditions.

(2) After the synthetic medium has been sown with yeast and incubated two days at 25° C., but before any flies have been put in the bottle, it has the same pH as the banana medium has initially. This movement in the acid direction is plainly due to the action of the yeast alone, and is in accord with the known effect of yeast growth upon the reaction of the medium.

(3) The pH of the medium becomes lower (*i. e.*, the reaction is more acid) the longer there are flies in the bottle. There is apparently an effect of the flies in this direction over and above that of the yeast. How this effect is produced is not clear from the preliminary experiments.

(4) After flies have been in the bottles two days the pH is practically the same whether the medium is banana or synthetic S–99. The experiments suggest that the medium comes, in about this length of time, to a state of equilibrium in respect of acidity.

III

These preliminary experiments indicated clearly the desirability of following the matter further. Accord-

[8] Brown, J. H., *Jour. Lab. and Clin. Med.*, Vol. 9, pp. 239–244, 1924.

ingly the following series of experiments was carried out.

Two series, of thirty-six one-ounce vials each, had the same amount of medium poured in each vial. In one series marked SA the medium was the synthetic S–99 described above. In the other series, marked BA, the medium was the standard banana agar already described. The S–99 bottles were lightly sown with dry yeast and incubated at 25° for two days before adding flies. At the end of this period fifty flies (twenty-five males and twenty-five females) were put into each of the seventy-two bottles. In the case of the BA series a light sowing of yeast was made with the flies. All the flies were of approximately the same age when put in, and were young (average age of flies at start 24 hours). Both the SA and BA series of bottles were put into a 25° incubator at the same time.

At the end of twenty-four hours four bottles were taken from the SA series, marked SA11, SA12, SA13 and SA14, and four bottles from the BA series marked BA11, BA12, BA13 and BA14. The flies were removed and the pH of the medium in each bottle was determined by the colorimetric method. Also the total acid per gram of medium was determined by titration with NaOH, using phenolphthalein as the indicator, and the results expressed as c.c. of N/10 NaOH equivalent to total acid per gram, using a standard solution of $NH_4H_2PO_4$ of pH 5.2 as the calibrating solution. At the end of the second day four more bottles were taken from each series, marked SA21, SA22, etc., and BA21, BA22, etc. These were treated in the same manner as the first day's set. This procedure was continued for nine consecutive days, or until all the bottles were used up. The dead flies were removed from the bottles and recorded each day.

The results are exhibited in Table 2. In this table the entries of the mean rows under the heading "mortality" are the number of deaths per bottle-day exposure to risk over the whole period, of the group concerned.

The same pH values from Table 2 are shown graphically in Fig. 1, and the total acid means in Fig. 2.

TABLE 2

pH, TOTAL ACID, AND MORTALITY IN SA AND BA SERIES

Day	Bottle number	pH		c.c. N/10 NaOH equivalent to total acid per gram		Mortality	
		SA series	BA series	SA series	BA series	SA series	BA series
Start*	01	4.0	5.3	0.44	0.25	.....	.....
	02	4.0	5.3	.35	.24	.....	.....
	03	4.3	5.3	.18	.22	.....	.....
	04	4.1	5.3	.25	.23	.....	.....
Mean at start	.....	4.1	5.3	.30	.24	.....	.....
1	11	4.2	5.3	.32	.28	0	0
	12	3.7	5.3	.49	.28	0	0
	13	3.7	5.3	.51	.28	0	0
	14	4.0	5.3	.32	.29	1	0
Mean 1 day	.....	3.9	5.3	.41	.28	0.25	0
2	21	3.5	5.2	.76	.30	0	0
	22	3.5	5.1	.69	.30	0	0
	23	3.6	5.1	.56	.30	0	0
	24	3.4	5.2	.83	.27	1	1
Mean 2 days	.....	3.5	5.15	.71	.29	.125	.125
3	31	3.5	4.8	.83	.43	0	0
	32	3.5	4.8	.67	.40	1	0
	33	3.5	4.9	.68	.41	0	0
	34	3.5	4.9	.88	.40	0	0
Mean 3 days	.....	3.5	4.85	.77	.41	.083	0
4	41	3.5	4.7	.98	.79	0	1
	42	3.5	4.7	1.24	.59	0	0
	43	3.5	4.7	1.07	.70	2	2
	44	3.5	4.7	1.68	.59	0	1
Mean 4 days	.....	3.5	4.7	1.24	.67	.125	.25
5	51	3.5	4.6	.82	1.50	1	1
	52	3.5	4.6	.98	1.40	1	0
	53	3.5	4.6	.75	1.26	0	0
	54	3.5	4.6	.96	.90	0	0
Mean 5 days	.....	3.5	4.6	.88	1.27	.10	.05

* That is, before flies are added. In the case of the SA bottles yeast had been incubated two days on the medium before these determinations were made, thus reducing the pH from its initial value when the medium is just made.

TABLE 2—continued

Day	Bottle number	pH		c.c. N/10 NaOH equivalent to total acid per gram		Mortality	
		SA series	BA series	SA series	BA series	SA series	BA series
6	61	3.5	4.8	1.07	1.84	7	3
	62	3.5	4.8	.86	1.00	5	1
	63	3.5	4.8	1.32	2.16	11	4
	64	3.5	4.8	.94	1.64	5	4
Mean 6 days	.....	3.5	4.8	1.05	1.66	1.17	.50
7	71	3.5	4.7	1.75	1.90	20	3
	72	3.5	4.7	1.13	2.34	7	2
	73	3.5	4.7	1.04	2.20	13	9
	74	3.5	4.7	1.14	2.36	13	4
Mean 7 days	.....	3.5	4.7	1.26	2.20	1.89	.64
8	81	3.5	4.8	1.56	3.53	37	8
	82	3.5	4.8	1.60	3.06	35	6
	83	3.5	4.8	1.80	3.62	29	1
	84	3.5	4.8	1.75	3.13	17	2
Mean 8 days	.....	3.5	4.8	1.68	3.33	3.69	.53
9	91	3.5	4.8	1.00	3.22	36	18
	92	3.5	4.8	.86	3.10	33	17
	93	3.5	4.8	1.27	1.77	50	34
	94	3.5	4.8	1.18	1.71	49	26
Mean 9 days	.....	3.5	4.8	1.08	2.45	4.67	2.64

From these data the following relations appear:

(1) The pH of the S-99 medium declines sharply during the first two days that the flies are on the medium, to a stable, buffered value of 3.5, at which point it remains throughout the remaining seven days covered in these experiments.

(2) The pH of the banana medium, starting considerably higher, declines steadily, but at a slower rate, during the first four days of the culture history after the addition of the flies to the bottle. The lowest point reached is 4.6. After the fifth day it rises slightly, show-

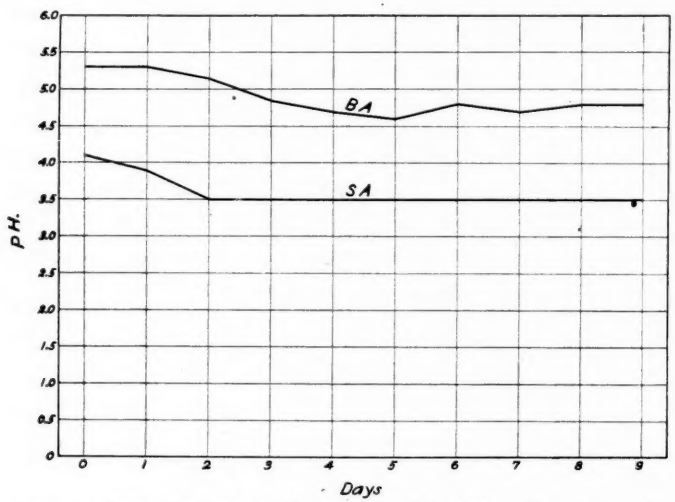

FIG. 1. Means pH values of the banana and the S-99 media on successive days in life of the culture.

ing a tendency to stabilize, presumably because of buffer action, at around 4.7 to 4.8.

(3) The total acid per gram of medium, as determined by titration with NaOH, using phenolphthalein as an indicator, increases in both the media compared up to the eighth day of the life of the culture. It then sharply declines on the ninth day. This increase in total acid follows, however, a distinctly different course in the two cases. In the case of the banana medium the increase is exponential; in the case of the S-99 medium it is somewhat irregular in its movement, but the general trend from the first to the eighth day is plainly *not* exponential, but is substantially linear. On the eighth day the total acid per gram of medium is almost exactly twice as great in the banana as it is in the S-99 medium. The equations fitted to the observations on mean total acid are as follows:

$$\text{BA series, } y = 0.183e^{0.355x} \qquad \text{(i)}$$

$$\text{SA series, } y = 0.331 + .148x \qquad \text{(ii)}$$

In these equations y denotes mean total acid per gram and x denotes time in days, it being understood that the graduation is between the limits 0 and 8 days only. The two curves suggest that the physico-chemical processes going on in the medium are different in the two media compared. In the banana medium the trend of acid production suggests at once that reaction is a catalyzed one, and that probably if the culture were continued long enough, and the factor of mortality, later to be discussed, could be kept more nearly constant, the curve of acid production would damp off asymptotically at the top like an autocatalyzed reaction. In the S–99 medium, on the other hand, the total acid per gram of medium appears to increase at a constant rate per unit of time over the period covered by the experiments. This points to a non-catalyzed type of reaction.

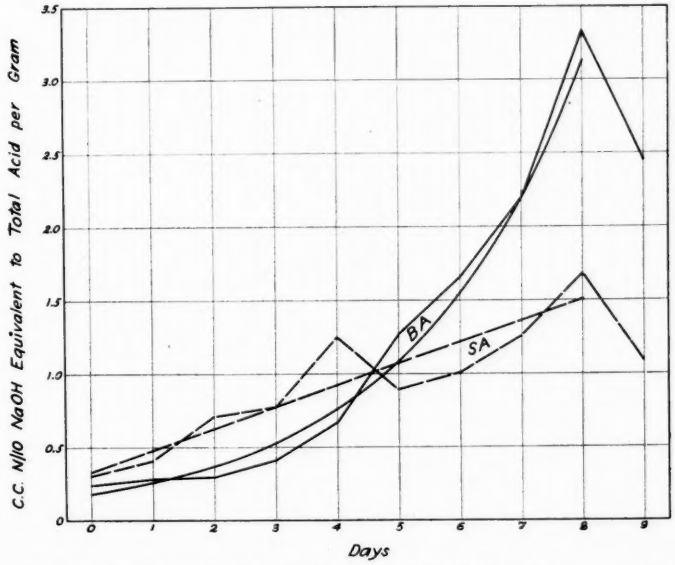

FIG. 2. Mean total acid in the banana and the S-99 media on successive days in the life of the culture. The smooth curves are the graphs of equations (i) and (ii) *infra*.

(4) The biological phenomena in the two sets of cultures were in certain respects markedly different. The fertility (number of offspring—whether larvae, pupae or imagoes—produced per female per day) was greatly higher in the S–99 than in the banana bottles. The presence of the greater number of larvae exhausted the food more quickly in the S–99 bottles. On the sixth day the mortality, for lack of food, became heavy in all the bottles, but more so in the S–99. The yeast had been all eaten up some time before this and starvation set in. The drop in the total acid per gram of medium on the ninth day, as compared with the eighth, is probably connected with the greatly reduced number of flies in the bottle, consequent upon the heavy mortality of the two preceding days. The much greater fertility observed in the SA bottles suggests that biologically this is a better medium on which to grow *Drosophila* than the banana. The results certainly indicate that it is not necessary to have fresh banana, or any other fruit, in the food medium in order to cultivate *Drosophila* successfully. The greater mortality rate in the former, in this experiment, does not contradict this view at all, because the mortality was due simply to starvation, resulting from the presence of too many flies for the amount of food in the bottle. Normally in the course of experimental work the flies, in such population densities as those here dealt with, would be transferred to fresh food every second day, and then the mortality from starvation would not appear.

SUMMARY

In this paper it is shown that the hydrogen-ion concentration of the medium increases with the age of the culture until a point of buffered equilibrium is reached, in cultures of *Drosophila melanogaster,* on both banana and a synthetic medium. The total acid per gram of medium increases throughout the life of the culture as long as relatively large numbers of flies are present. The bio-

logical relations suggest that the synthetic medium here described is better suited to practical fly husbandry than is the standard banana medium, and demonstrate that the presence of fresh banana, or any other fruit, is in no wise biologically essential as a constituent of the food medium in the culture of *Drosophila* as far as concerns the processes of reproduction.

CULTURE MEDIA FOR DROSOPHILA. II. A NEW SYNTHETIC MEDIUM AND ITS INFLUENCE ON FERTILITY AT DIFFERENT DENSITIES OF POPULATION[1]

RAYMOND PEARL, AGNES ALLEN, AND W. B. D. PENNIMAN

I

THE results reported in the preceding paper in this series showed that *Drosophila* could be successfully cultivated on an entirely artificial medium, in which no natural fruit product was present, and which had a much higher degree of acidity, as indicated by the pH, than has the standard banana medium in common use. There are certain obvious practical advantages in carrying the acidity of the culture medium to as high a point as possible, having regard to normal behavior and vitality of the flies themselves, because if the pH of the medium can be brought below the limit for the growth of moulds and bacteria which occasionally contaminate cultures of *Drosophila,* the deleterious effects of these other organisms in experimental work will be automatically avoided.

With this idea in mind, various still more acid synthetic media were tried. It is the purpose of this paper to report upon one of these, S–101, which has proved to date to be the best of the lot, and in every respect so far tested greatly superior from a biological standpoint to the standard banana medium.

[1] From the Institute for Biological Research of the Johns Hopkins University.

The composition and mode of preparation of this new synthetic medium are as follows:

SYNTHETIC, S–101

Solution A.	Cane sugar	500 gms.
	$KNaC_4H_4O_6 4H_2O$	50 "
	$(NH_4)_2SO_4$	12 "
	$MgSO_4 7H_2O$	3 "
	$CaCl_2$	1.5 "
	H_2O to make 3000 c.c. of solution	
Solution B.	Agar-agar	135 gms.
	Tartaric acid ($C_4H_6O_6$)	30 "
	KH_2PO_4	6 "
	H_2O to make 3000 c.c. of solution	

Melt the agar thoroughly in the water with heat, add the salts, and for the medium to be used in the fly bottles, mix equal parts of solutions A and B. For some kinds of work it has proved desirable to have the final food a little less stiff, in which case a small amount of agar is used, without changing the composition otherwise.

This medium has a pH when freshly made, cooled, and the agar set, of approximately 3.7. As the flies live upon it the pH falls to a value of 3.0, or in some cases even lower.

II

This new medium has been tested in a great variety of ways in the laboratory. It has proven so satisfactory that all our *Drosophila* stocks are now carried on it, as a routine. On account of its high acidity there is practically never any contamination of the cultures by troublesome bacteria. In particular *Bacillus subtilis*, which can make a great deal of trouble on the standard banana medium described in the preceding paper in this series, never gets a foothold on this medium S–101. Some moulds will grow on it, but the trouble from this source in routine *Drosophila* work is greatly reduced by the use of this medium.

In order to test the influence of this medium on the fertility of *Drosophila* a series of experiments was performed, according to the following plan:

Using wild type flies of a line-bred strain, half pint milk bottles were set up according to the scheme which follows:

No. of bottles	Number of parent flies put in each bottle at start	Initial density (flies per bottle)	Medium
4	1 pair	2	Two bottles at each specified density were filled to the depth of ¾ inch with standard banana medium, and lightly seeded with yeast.
4	2 pairs	4	
4	4 "	8	
4	8 "	16	The other two bottles at each specified density were filled to the same depth with S–101 medium, lightly seeded with yeast and incubated at room temperature two days before the flies were put in.
4	16 "	32	
4	32 "	64	

From these conditions it is seen that volume of food, and surface area of food were the same in all bottles. Density of population in the strict sense was selected as the controlled independent variable in these experiments.

All the bottles were placed into the same incubator operating at 25° C. and carried at that temperature throughout the experiment. Each day each bottle was examined, any dead flies removed and a record made of the date of death and the sex of the fly. At the end of eight days the parent flies were removed from the bottles, before any of their progeny had emerged as imagoes. The bottles themselves were then continued in the incubator and counts of the progeny emerging as imagoes made each day for a period of eight days after the first progeny fly emerged.

There is only one other point regarding the technique of these experiments which wants further discussion. It relates to the seeding with yeast. The new synthetic medium S–101 is seeded two days in advance and the banana medium only at the time of adding the flies, for the following reason: The S–101 medium is so acid as appreciably to retard the growth of yeast, while on the banana medium yeast grows promptly and luxuriantly. If the S–101 medium is seeded with yeast at the same time the flies are added, they will in the higher densities eat up all the seed yeast before it gets well started to grow, and then the flies suffer later for lack of food. By

following the procedure here outlined and starting the yeast on the synthetic medium two days in advance at room temperature, both banana and S–101 are in the same condition relative to abundance of food in twenty-four hours or less after the flies are added.

III

The results respecting fertility in these experiments are set forth in Tables 1 and 2. In these tables are recorded the initial population densities at which each bottle started (number of flies per bottle, all bottles being the same size and containing the same volume and surface area of food, and volume of air space above food); the mean density of population over the eight-day period, which figure takes into account the number and time of the death of all the parent flies; the number of female-days, being the sum over eight days of the number of female parent flies in each bottle each day; the absolute number of progeny flies produced in eight days forward from the time of emergence of the first progeny fly; the number of progeny produced per female per day over the eight-day period, got by dividing the figures in the fifth column by those in the fourth column; the total number of deaths among the parent flies in the eight-day period; the death rate per one hundred exposed to risk over the eight-day period, got by dividing the total deaths (x 100) by the number of flies exposed to risk of dying at the beginning of the period.

In this paper the term "fertility" is used to mean the number of adult progeny flies (imagoes), expressed either as an absolute number, or as relative to the number of mated females per day. The question of the influence of the new synthetic medium upon fecundity (the number of eggs laid per mated female per day) or upon the production of larvae is not here discussed. The important problems presented by these other elements in the reproductive process are left for discussion at a later date.

Let us consider first the total number of progeny flies produced per bottle in eight days, in each of the two

TABLE 1

THE PRODUCTION OF PROGENY OF DROSOPHILA MELANOGASTER ON THE SYNTHETIC MEDIUM, S–101

Bottle No.	Initial density of population	Mean density over 8-day period	Total female days	Total progeny in 8 days	Progeny per female per day	Total deaths in 8 days	Death rate over 8-day period
1	2	2.00	8	294	36.75	0	0
2	2	2.00	8	257	32.13	0	0
Mean	2	2.00	16	551	34.44	0	0
3	4	4.00	16	371	23.19	0	0
4	4	3.80	12	332	27.67	1	25.0
Mean	4	3.90	28	703	25.11	1	12.5
5	8	7.80	32	348	10.87	1	25.0
6	8	7.80	32	362	11.31	1	25.0
Mean	8	7.80	64	710	11.09	2	12.5
7	16	15.50	60	300	5.00	2	12.5
8	16	16.00	64	312	4.88	0	0
Mean	16	15.75	124	612	4.94	2	6.25
9	32	31.40	124	204	1.65	2	6.25
10	32	31.40	123	264	2.15	3	9.38
Mean	32	31.40	247	468	1.89	5	7.81
11	64	60.56	238	257	1.08	7	10.94
12	64	62.75	255	242	1.05	5	7.81
Mean	64	61.66	493	499	1.01	12	9.38
Grand totals and Means	.....	.....	972	3543 295.25*	3.65** 13.14†	22	8.73

* Mean absolute number of progeny in 8 days per bottle, unweighted.

** Mean progeny per female-day, each bottle weighted according to its female-days.

† Mean progeny per female-day per bottle, unweighted.

TABLE 2

THE PRODUCTION OF PROGENY BY DROSOPHILA MELANOGASTER ON STANDARD BANANA MEDIUM

Bottle No.	Initial density of population	Mean density over 8-day period	Total female days	Total progeny in 8 days	Progeny per female per day	Total deaths in 8 days	Death rate over 8-day period
13	2	2.00	8	188	23.50	0	0
14	2	2.00	8	185	23.13	0	0
Mean	2	2.00	16	373	23.31	0	0
15	4	4.00	16	183	11.44	0	0
16	4	3.30	10	197	19.70	1	25.0
Mean	4	3.65	26	380	14.62	1	12.5
17	8	7.50	26	201	7.73	4	50.0
18	8	7.80	40	234	5.85	1	12.5
Mean	8	7.65	66	435	6.59	5	31.25
19	16	15.40	58	162	2.79	3	18.75
20	16	15.80	62	191	3.08	5	31.25
Mean	16	15.60	120	353	2.94	8	25.0
21	32	30.90	114	188	1.65	16	50.0
22	32	31.10	112	75	.67	7	21.9
Mean	32	31.00	226	263	1.16	23	35.9
23	64	61.80	240	131	.55	23	35.9
24	64	63.25	254	119	.47	8	12.5
Mean	64	62.53	494	250	.51	31	24.2
Grand totals and Means	.....	.....	948	2054 171.17*	2.17** 8.38†	68	26.98

* Mean absolute number of progeny in 8 days per bottle, unweighted.

** Mean progeny per female-day, each bottle weighted according to its female-days.

† Mean progeny per female-day per bottle, unweighted.

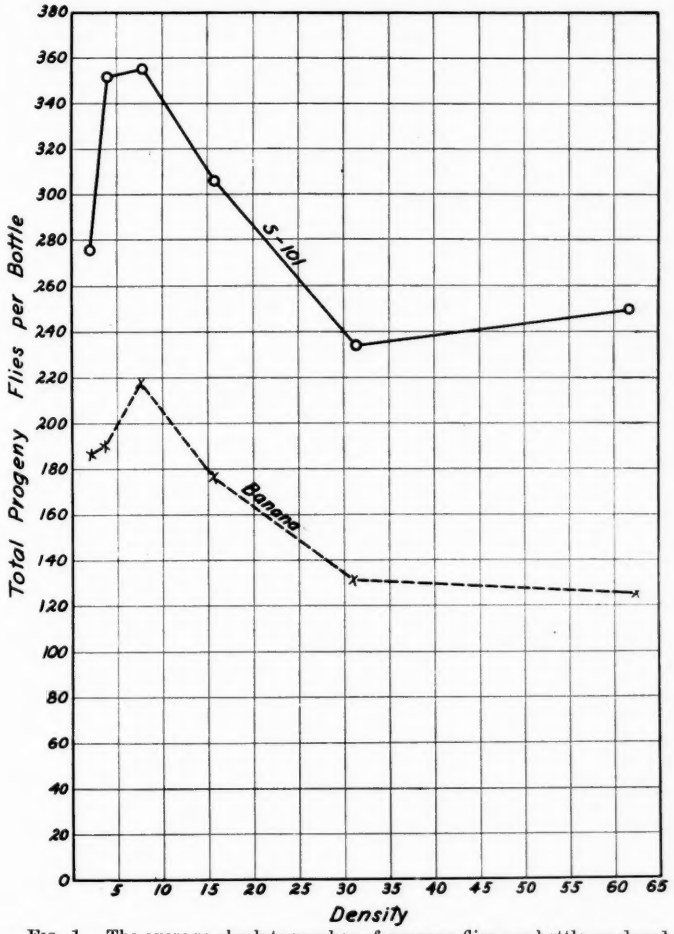

FIG. 1. The average absolute number of progeny flies per bottle produced in the S-101 series (solid line) and the banana series (broken line).

series, on the basis of the data set forth in Tables 1 and 2. In Fig. 1 the total absolute number of progeny flies per bottle (the average of the two bottles in each density group) are plotted as ordinate, and the mean density of population over the eight-day period as abscissa.

It is at once obvious from this diagram, and the data in Tables 1 and 2 upon which it is based, that many more progeny flies (imagoes) per bottle were produced on the

synthetic, S–101 food, than on the standard banana medium, the total volume and surface area of food being the same in the two series. This was true of all population densities. The absolute progeny productivity curves rise rather sharply from values of 275.5 and 186.5, respectively, at the initial density of 2, to a high point at initial density 8. They then fall off rapidly until the bottles of

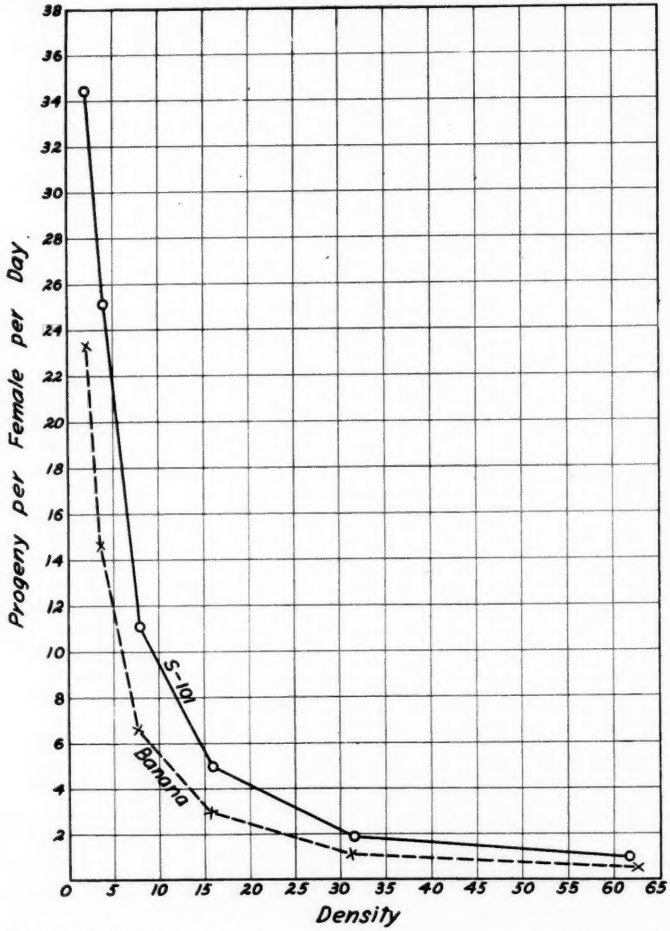

FIG. 2. Progeny per female per day on the new synthetic medium S-101 (solid line) and on the standard banana medium (broken line).

initial density 30 are reached. In the synthetic medium (S–101) the total absolute progeny produced per bottle is a little higher at initial density 64 than at initial density 32, whereas in the case of the banana series the absolute productivity value at initial density 64 is slightly lower than at initial density 32. It is evident from these figures and the diagram that the production of progeny (fertility) is greatly increased on the new synthetic medium as compared with the old standard banana.

The same thing is shown if the more precise method of expressing fertility in terms of progeny per female per day is adopted. This method gives the curves shown in Fig. 2.

It is again obvious that the parent productivity, as measured by imagoes produced per female per day, is higher on the S–101 medium at all densities than on the standard banana medium. The relative amount of this excess is shown by the following percentage figures, which are the percentages which the differences between the two series are of the banana figures.

PERCENTAGE INCREASE IN FERTILITY (PROGENY PRODUCED PER FEMALE PER DAY) ON THE SYNTHETIC MEDIUM S–101, AS COMPARED WITH STANDARD BANANA MEDIUM

Initial density	Percentage increase
2	47.7
4	71.8
8	68.3
16	68.0
32	62.9
64	98.0

There can be no doubt that the production of progeny, however measured, is much higher on the synthetic medium than on the standard banana.

It may be noted, although it is not our purpose to discuss this point especially in this paper, that the results of these experiments agree closely with those obtained by Pearl and Parker[2] in their earlier study of the effect of

[2] Pearl, R. and Parker, S. L., Proc. Nat. Acad. Sci., Vol. 8, pp. 212–219, 1922. See also Pearl, R., "The Biology of Population Growth," New York (Alfred A. Knopf), 1925.

density of population upon fertility in *Drosophila,* in which work banana medium was used.

The difference between the two series in respect of mortality is quite as striking as that just shown in fertility. Whereas in the eight days only 8.73 per cent. died of the 252 flies exposed to risk of dying in the S–101 bottles, 26.98 per cent. of the 252 flies exposed to risk over the eight days in the banana bottles died. The mortality was relatively three times as great on the banana medium as on the synthetic. The mortality on the banana medium was heavier in this experiment than is usual in our work, so that it would be unwarranted to conclude that generally the new synthetic medium will show as great a superiority in respect of mortality as it did in this particular case. Yet, in spite of this necessary reservation, we feel reasonably certain from other experience with this new medium that there will generally be found to be a smaller mortality of the flies kept on S–101 medium than of those kept on standard banana medium. Experiments are now in progress from which we expect to be able to present much more detailed figures covering this question of relative mortality on the two media.

SUMMARY

In this paper is described the composition and method of making a standard synthetic medium for the laboratory cultivation of *Drosophila melanogaster.* It is shown that this medium is greatly superior to the banana medium commonly used for this purpose in respect of both the fertility and the mortality of the flies kept on it. The range of superiority in respect of fertility is at different density of population from about 48 per cent. at the lowest to 98 per cent. at the highest densities experimentally reported here. The general experience of the laboratory with this medium, which frees experimental work on *Drosophila* from the incubus of the highly variable banana, shows it to have other points of superiority besides those discussed here.

THE STORING HABIT OF THE COLUMBIAN GROUND SQUIRREL[1]

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DURING investigational work relative to the life history of the Columbian ground squirrel (*Citellus columbianus columbianus*) by the writer for the Washington Experiment Station, abundant opportunity was found for making observations on the question of storing as practiced by this rodent.

By the term store we mean the provisions which might be stored by the squirrel for future use during estivation, hibernation or other periods. The question as to whether or not this species of ground squirrel stores food for the winter or other seasons has been one of much doubt and the basis for considerable argument. It might be said that in the case of the 136 dens dug out directly for hibernating squirrels and twenty-seven summer and brood dens dug during the years of 1910 to 1915, inclusive, in no case was a store found in a summer den and it was observed that only a part of the hibernation dens possessed them. The store, which is usually small, is commonly placed in the back of the hibernation cell, in the mulch, with the squirrel between it and the entrance.

Out of forty-one hibernation nests of wild squirrels carefully examined twenty-one contained a store. In only one case was a store found definitely in the den of a female squirrel. Twelve of the other nests in which the squirrels were taken with the nest were those of males, most of them adults, as will be seen from the following.

[1] Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 128, College of Agriculture and Experiment Station, Pullman, Washington.

Date	Den Number	Sex	Weight
Dec. 23, 1912	1	Male	615 grams
Mar. 7, 1914	3	"	609 "
Dec. 13, 1913	1	"	594 "
Feb. 21, 1914	1	"	579 "
Mar. 3, 1915	17	"	557 "
Mar. 7, 1914	2	"	536 "
Mar. 1, 1915	1	"	522 "
Feb. 22, 1913	1	"	571 "
Mar. 6, 1914	1	"	517 "
Mar. 1, 1915	8	"	435 "
Feb. 20, 1914	2	"	407 "
Aug. 16, 1911	1	Female	434 "

The average weight of an adult male is 517 grams. Of the remaining eight nests, six fresh but unoccupied at the time of taking; one was occupied by a male squirrel, the weight of which was not taken, and one was occupied by a female squirrel taken from Den 1 on Dec. 13, 1913. This female weighed 371 grams, and in her nest was found a trace of a store in the form of few pepper-grass pods (*Alyssum alyssoides*), but no seeds. It looked as if this might have been accidental and that there was in reality no store in this nest.

These data serve to point pretty strongly to the fact that storing is done chiefly by the male squirrels. This might be accounted for by the fact that *the old males appear a week or ten days earlier than the females and young of the previous year,* and at a time when the weather is frequently wintry and unsettled, with the ground covered with snow and food hard to find, as in the spring of 1917.

In the connection of all the investigation done with squirrels in captivity only one was found storing, and this was a male weighing 451 grams. He hulled some oats from a sheaf in the yard and stored it in his hibernation nest.

The fact that the stores are found chiefly in the cells of the males would tend to point to the fact that the male and the female squirrels each go back to their respective cells year by year or else females would have been found in cells occupied by males of a previous year, where they

might have gone in on an old unused store. The size of the squirrel probably determines the cell they occupy. It is possible, too, that these hibernation cells may be cleaned out each summer before building a new nest.

That the store is placed in the hibernation cell by the owner and occupant and not by such guests as the pocket gopher and mice is strongly evident, owing to the fact that stores were not found in other nests of summer dens or in other parts of the dens. When caches of the pocket gophers were found by accident they were not in the squirrel den proper but in chambers near one of the small holes of the pocket gopher.

It must not be supposed that all old male squirrels store food for the spring, for five nests of these and younger males were found quite destitute of store. Also the hibernation nests of seven females were found with no store.

There are many indications that the store is not always used and that it is probably of use chiefly as a safeguard in case of a sudden burst of storm or a long protracted stormy season after the squirrels have come from hibernation. It has been thought that these stores may be used as a community cache, though no direct data substantiate this.

On February 27, an old unused hibernation cell and nest was found in which was a quantity of shelled wheat, which was matted in the bottom of the cell, and which had been sprouted, probably by the warmth of a previous summer's heat. Also in the case of Den 1 of March 1, 1915, in the hibernation nest of which were some small fresh potatoes and, what was more remarkable, some dried potato skins, suggesting occupation in a former year. Again on March 3, 1915, while digging for a squirrel we ran into an old unused cell containing old oats. On February 21, a quantity of old oats was found in the hibernation nest No. 1, again suggesting use in former years.

In the case of the deserted caches it would indicate that the store was given up in preference to green food if the weather were good, at the time of their coming out, as it was in the spring of 1915, when the six deserted stores were examined. That the squirrels rely upon their store for spring food is shown by the squirrel taken from his nest February 22. This specimen had not left his hole, which had been tunneled through the snow, yet he had food in his stomach, and one of the potatoes was almost entirely and freshly eaten. Old skins of potatoes were also found in the nest, suggesting that it had been used in previous years.

This lack of providing the nest with a store is in strong contrast to the abundant storing of the Douglas ground squirrel (*Citellus grammurus douglasii*) brought from the Rogue River, Oregon, in 1911. These squirrels would store all the food given them if not needed for immediate use, even to the remains of their own dead which they had previously killed and partly devoured.

They would eat in the nest during the winter. Those under observations in the cabin did not go into hibernation but remained warm all winter, though staying in the nest closely. A squirrel in one of the wild dens in Yard 7 did not come out between fall and early spring, having stored abundant food material before winter came on. In addition, much data relating to storing were taken in connection with the food habits in which thirty-six squirrels were collected from Pullman and nearby territory for the especial purpose of stomach contents examination. In most cases these squirrels were shot and as a result the normal condition of the cheek pouches would be learned. The results of this inquiry as relating to the cheek pouches and related storing is as follows:

Again on July 10, a young female, weighing 454 grams, was shot on the roadside by a winter wheat field. She had twenty-five grains of wheat in her cheek pouches.

On July 18, a female, weighing 432 grams, was taken near a feeding rack in which was some wheat hay, or

wheat sheaves such as are used in this locality for hay and which have been cut green and in which the grain is shriveled. This squirrel had a single grain of wheat in her cheek pouches.

While trapping squirrels, August 7 to 9, for use in the hibernation yards, a young female was taken midway in a little stream gulch on a path running from an old sodded roadside to a field of winter wheat. She had a few grains of wheat in her cheek pouches. Although caught in a steel trap she still retained the grain in her cheek pouches when taken from the trap.

These are all the cases in the entire investigation when food was found in the cheek pouches of these squirrels. The data do not clearly harmonize with the store, sex and age of the squirrels found storing in hibernation as noted above, and suggest a further use for the cheek pouch, namely, the collecting of grain at a distance from the nest while gleaning to be used immediately as food when the squirrel returns to his den; and not for storing. This idea would be substantiated by observations made in captivity to the effect that squirrels usually carry foraged food to the entrance of a burrow before stopping to eat it, this being a precautionary measure against the enemy. On one occasion a squirrel in the observation yards was observed picking up scattered grain. After a while she ran over the entrance of a burrow and appeared to be eating what she had gathered. I have been told, too, that squirrels poisoned by picking up scattered grain were found to have some of the grain in their cheek pouches, which viewed in this light would not necessarily mean that they were going to store it. On the other hand, the fact that they were killed by it would tend to show that they were picking it up for immediate use and not for the hibernation store.

Composition of Store

A list of the seeds and bulbs found stored in forty-four hibernation nests and the frequency of use is as follows:

Name of plant used	Number of times used
Collinsia tenella	9
Potato	8
Wheat	5
Oats	3
Alyssum alyssoides, Pepper-Grass	2
Erythronium grandiflorum, Dog's-tooth Violet	2
Avena fatua, Wild Oats	2
Barley	2
Rosa Nutkana (? sp.), Wild Rose	2
Claytonia linearis, Spring Beauty	2
Rumex, sp., Dock	1
Allium acuminatum, Wild Onion	1
Tellima tenella or *parviflora*, Baby Face	1
Apple	1
Bromus, sp., Brome Grass	1
Polygonum convolvulus, Wild Buck-wheat	1
Zygadenus venenosus, Death Camas	1

Of all the seeds used *Collinsia* seemed to be selected most frequently and used by far in the greatest abundance, as will be noted in the cases of Den 2 of February 21, where an enormous quantity was found, and Den 2 of March 5, when 950 cc of the pods were found mixed with dust. Another nest was found with 190 cc of the ripe seed pods of this plant. Those pods may have been the accumulation of some years. Of bulbs or tubers the potato was used most often, the smaller ones, .5 to 1 inch being selected. One nest contained 101 bulbs of *Allium acuminatum*.

A very surprising observation was that of the discovery of the bulbs of the Death Camas in one store. This bulb is regarded as very poisonous to most animals. The apple, wild rose, spring beauty and wild buckwheat were used very sparingly.

Perhaps the chief reason for using these forms of vegetation would be their early maturation. *Collinsia* is one of the first plants to ripen and is very abundant on shallow soil on southern exposures. Wheat, barley, oats and wild oats may be had from unthreshed heads of the

previous year or from seeds scattered over the soil. Bulbs, *e.g. Allium,* may be had, many of them in a ripe state at the time needed for estivation. The apple seeds found might have been taken from apples of the previous year which when covered by snow all winter remain good well on towards the summer of the following year. A windbreak row of apple trees was near the den from which the seeds were taken. It is interesting to note that wild seeds seem to be preferred as a store in the hibernation nest.

Finally, it might be said that while this species of *Citellus* is not an active storer of food, except as has been shown, it should not be taken as a representative of the genus in the matter of winter provision. Undoubtedly other representatives of the genus, living under conditions of varied climate, will have their own methods of providing for their special periods of adversity.

CRUCIAL EVIDENCE FOR ANTARCTIC RADIATION

PROFESSOR LAUNCELOT HARRISON
UNIVERSITY OF SYDNEY

RECENT controversy in THE AMERICAN NATURALIST between Professor Maynard M. Metcalf and Dr. G. K. Noble upon the origin and dispersal of the families of Salientia and upon the value of opalinid parasites in the study of this problem has touched upon a question which is of outstanding interest to the Australian zoologist, namely, the dispersal route of the Australian fauna. Australasian zoologists in general favor an Antarctic radiation for a considerable proportion of that fauna, including especially, amongst vertebrates, the marsupials and hylid and leptodactylid frogs. In recent years, however, many attacks have been made upon the idea of Antarctic radiation, of which the most important are, perhaps, those of Matthews, Tate Regan, Andrews and Noble. I have replied to some of this criticism recently (Harrison, 1924). I write now upon two grounds; first, to make reply to some recent remarks by Noble (1925), and secondly, as an independent originator of the host-parasite idea supported by Metcalf (Harrison ,1915, seq.), to offer some further parasitic considerations which seem to me to have a bearing on the question at issue. I hasten to add that I am not sufficiently a student of the Salientia to be competent to adjudicate upon Noble's recent reclassification of the group, nor have I an adequate knowledge of the Opalinidae such as would justify my discussing Metcalf's broad conclusions. It is only where these workers impinge upon Australasian matters that I desire to offer my opinion. I should like to add further that the ultimate fate of the Antarctic Continent theory will be settled by facts and by just inference from those

facts, but not by mere argument. Doubtless enthusiasts are very easily led to press their inferences a little beyond their facts and doubtless specialists usually base their views upon a body of evidence much larger than that they publish, but dialectic is not proof.

Noble has merged the *Leptodactylidae* into the *Bufonidae,* and writes (1925, p. 270);—"When this is done it is found that the leptodactylids, or, as we now call them, bufonids, have a world-wide range, and lend no support to the theses expressed by Metcalf." While such a statement may be broadly true, it is not sufficiently true to justify the use which Noble makes of it. I repeat my former answer to a similar statement (Harrison, 1924, p. 257):

> Noble has united the Bufonidae and Leptodactylidae, calling them all Bufonids. This action has not had time to draw upon itself the requisite criticism which will decide whether it be well founded or no. It does, however, enable Noble to claim a continuity of Bufonidae from Asia into Australia which, on closer analysis, does not exist. Typical northern Bufonids of the genus *Bufo* have not been able to penetrate to the east or south of Celebes. The Australian Leptodactylids, the essential characters of which are not in any way altered by calling them Bufonids, have not succeeded in penetrating to the north or west of Papua, in which island they are represented by one or two species only; and their closest affinities would still seem to lie with South American forms.

Noble's allegedly continuous bufonids are thus absent from the islands between Celebes and Papua, the latter word being used in its original sense for the whole island of New Guinea. As a matter of fact, the gap is really much wider, since the genus *Bufo,* as far as can be judged from available evidence, is a comparatively recent migrant to the south and east. Taking the gap, however, at its narrowest actual limits, no continuity exists across it. In many places so inconsiderable a hiatus might be of no great importance, but this particular gap includes that very important transition zone, roughly bounded by the "Lines" of Wallace and Weber, which so sharply demarcates the Asiatic from the Austro-Malayan fauna. This is classic ground, and it can not be supposed that Noble

is ignorant of it. *Bufo* has no near relatives on the Australasian side, and the Australian "bufonids" have no near relatives on the Asiatic side, so that it becomes a little difficult to be patient with Noble's claim of continuity.

Metcalf may be left to fight his own battles as regards opalinids in general, but in so far as these concern the Australian problem, I can not refrain from comment on Noble's "criticism" of what appears to me to be a most important piece of evidence. Noble writes (1925, p. 269), "In regard to the leptodactylids, Metcalf has nothing to add, save that certain opalinids and these frogs happen to be found in the southern hemisphere." One would imagine from such a statement that opalinids and frogs were quite independent of one another, instead of which these parasites, with a few inconsiderable exceptions, are entirely parasitic in, and dependent upon, their frog hosts. Moreover the opalinid genus *Zelleriella* is found only in Australia and southern America, and in Australia occurs exclusively in leptodactylids. If Australian "leptodactylids" and Asiatic "bufonids" are genetically continuous, as claimed by Noble, it would appear to be his duty to offer some explanation of the means by which *Zelleriella* reached Australia, since there is no evidence of the occurrence, past or present, in any palearctic frog; and also of its non-occurrence in Palearctic "bufonids." The absence of multi-nucleated opalinids from Australia in general, and from Australian hylids in particular, though they are present in Asiatic hylids, is another fact that demands adequate consideration at the hands of those who hypothecate a northern center of dispersal for the group. These two conditions taken together constitute, to my mind, a very potent, if not conclusive, argument for dispersal from the south. but this, as I have said before, is Metcalf's affair. I would, however, remind critics that opalinids must have evolved in, and *pari passu* with, frogs, and have not an independent zoogeography of their own, as Noble seems to imply.

Noble would explain the hiatus in southeastern Asia, which he admits for hylids and which holds equally for "leptodactylids" and marsupials, by problematic extinctions in that region. There might be some justification for this, could any good grounds for suspecting such wholesale extinctions be adduced. The available evidence, however, indicates that Malaysia has suffered few and inconsiderable climatic vicissitudes since Mesozoic time, and that the physical environment in this region has always been eminently suitable for the persistence and survival of creatures such as marsupials and frogs. Moreover, even the very marked progressive aridity of central Australia since the Cretaceous has not sufficed to extinguish hylid and leptodactylid frogs, which still persist, and have developed remarkable adaptive habits in response to changed climatic conditions. The absence of marsupials, hylids and leptodactylids from southeastern Asia is a coincidence far too important to be dismissed by unsupported suppositions about problematic extinctions. Advocates of northern dispersal appear to base their claims upon the known tertiary migrations of placental mammals. Seeing that these penetrated into the Malay Archipelago, but were unable to reach Australia, it is curious that a thesis which thus breaks down as far as Australia is concerned should be pressed for other groups without any evidence in support. A considerable proportion of the Australian fauna has reached Australia from the north, including ranid and gastrophrynid frogs, but the evidence for these invasions is unmistakable. There is no positive evidence whatever that marsupials and hylid and leptodactylid frogs entered by a northern path.

There is equally no positive evidence that they came in by the south. But if it can be proved that migration was possible by the south, and I believe I have a convincing instance to offer, then, in view of the preponderant distribution of the three groups in question in South America, a southern migration route becomes infinitely more

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probable. Since the cogency of my instance will depend upon the view taken of the host-parasite relation, I should like to stress the importance of this general concept. It has been advocated independently by a number of workers on different parasitic groups, Metcalf's statement for the opalinids being the latest. My own experience is chiefly concerned with lice, and, since my first announcement of my belief in the value of these parasites for the study of host phylogeny in 1911, all my studies have tended to confirm that belief. The Mallophaga of birds, more especially the Ischnoceran section, afford very precise indications of the relationships of their hosts. As an example of the kind of thing that happens quite frequently, I was recently examining the parasites of the Steganopodes. I found that, while the cormorants, gannets, pelicans and frigate-birds were obviously closely related, the tropic-birds of the genus *Phaethon* were, judged by their parasites, wrongly included in the group and should be placed with the terns. On my mentioning the matter to Mr. Tom Iredale, he informed me that he had reached the same conclusion on morphological grounds and added that Dr. James Waterston, of the Imperial Bureau of Entomology, London, also a student of Mallophaga, but one who had been rather dubious about the views I held as to host relationships, had become converted and had given him considerable help in clearing up doubtful relationships amongst the limicoline birds from a study of their mallophagan parasites. To argue here the whole case for the value of parasitic evidence would take an undue amount of time and space, but I am firmly convinced of the great importance of this kind of evidence, which has hitherto received very inadequate consideration. The Mallophaga scarcely help my present argument, but it is of interest to note that none occur on monotremes, only Amblycera on marsupials and on a few South American rodents (these I have supposed elsewhere (1922) to be stragglers from marsupials) and only Ischnocera on placentals in general. Marsupials in Aus-

tralia and South America carry the same kind of mallophagan parasites, which differ from those of all other mammals, but this fact does not help in the matter of migration routes.

The fresh-water crayfishes, however, and their temnocephaloid parasites, seem to me to offer the crucial evidence for which I have been seeking. The crayfishes themselves have been the basis for a series of discussions at the hands of Huxley, Ortmann, Geoffrey Smith and Matthew. There is agreement that those of the northern hemisphere (Potamobiidae) differ widely from those of the southern hemisphere (Parastacidae), and that these families make contact only in Central America, crayfish being otherwise absent from the tropics and from Africa. Smith would derive the families independently from different marine ancestors. Matthew would derive the parastacids from primitive potamobiids. Parastacids are found in South America, New Zealand, Australia (including New Guinea) and Madagascar, a distribution that is common to some other animal groups. Matthew supposes that these southern land masses received their crayfishes by separate migrations of northern potamobiids, or rather, of the more primitive ancestors of these, and would derive the Mascarene crayfishes through hypothetical African ancestors.

The Temnocephala are parasitic upon crayfishes in Central and South America, as well as upon other fresh-water decapods and tortoises in the latter continent; upon crayfishes in New Zealand and Madagascar; and upon crayfishes and other fresh-water decapods in Australasia. They are preponderantly parasitic on crayfish hosts. They extend into the Austro-Asiatic tropics upon fresh-water crabs, and into the American tropics upon crayfish; otherwise they do not occur in, or north of, the tropics. No marine temnocephaloids are known. The group is commonly considered as a separate order of monogenetic trematodes; but there is a good deal of evidence which suggests an independent origin from the rhabdocoele turbellarians.

The available evidence seems to indicate that the temnocephaloids are a fresh-water group, with no suggestion for marine origin. They have somehow become distributed over four southern land masses—South America, Australia, and Madagascar, but not Africa. The Parastacidae have precisely the same general distribution, but their associated parasites seem to have worked north as far as the Philippines, in the Austro-Asiatic tropics, upon fresh-water crabs; and as far as Mexico in the American tropics, where one species has succeeded in establishing itself upon a potamobiid. Positive evidence for dispersal from north to south is absent. Except for the occurrence of a species upon *Cambarus digneti* of Mexico, which I think must be admitted as of recent date, they do not occur upon the Potamobiidae.

The acceptance of Matthew's hypothesis of four separate dispersal streams of crayfishes from the northern hemisphere potamobiids into Madagascar (through Africa), Australasia, New Zealand and South America implies:

(3) The presence in the past of temnocephaloids upon northern potamobiids, for which there is no evidence.

(2) The extinction of both crayfishes and temnocephaloids in Africa, where there is no evidence that either ever existed and no obvious or plausible reason as to why either or both should have become extinct.

(3) The general distribution in the past of both crayfish and their parasites in the tropical belt, for which again there is no positive evidence. Moreover, since opportunity has been afforded for the southern crayfish to migrate into the tropical belt, and since they have not done so to any marked degree, it would seem that the tropics do not afford a congenial environment for crayfishes.

(4) The extinction of temnocephaloids upon Asiatic and North American potamobiids, for which there is no evidence, and which should not, I think, be assumed without some justification or explanation.

These considerations seem to me to rule out Matthew's hypothesis completely. If the parastacids have been derived from potamobiids, the only possibility seems to be that such derivation took place in America, and that the parastacids, as such, first appeared in South America, and must have reached the other southern land masses by

a southern route of dispersal, carrying their temnocephaloid parasites with them.

If on the other hand, we accept Geoffrey Smith's suggestion that potamobiids and parastacids were independently derived from separate marine ancestors, unless we assume that the marine ancestors of the latter possessed temnocephaloid parasites, an assumption for which there is no warrant, we must arrive at much the same conclusion. Multiple origin and convergent resemblance are ruled out by the common occurrence of both host and parasite groups. These might be argued for one or other, but to apply them for both is asking too much of coincidence. Therefore, though it is open to assume that parastacids came into being either in Australia, or in South America, the problem of getting them to the other land masses is still one of southern dispersal.

If, in place of problematic and improbable extinctions, we accept recognized facts, there is an actual, not hypothetical, antarctic continent, known to have had a temperate climate and flora from Jurassic to Miocene and therefore certain to have had a fauna. This fauna has become extinct, but here the extinction can be easily and justifiably explained. This continent comes into fairly close relationship geographically with the southernmost points of South America, New Zealand and Australia. Faunistically, there is a vast body of evidence indicating closer connection. The Wegener hypothesis, if it ever prove acceptable to geologists, would explain Australian problems admirably, but this I can not discuss here. Geologists have certain objections to the amount of alteration necessary for the formation of land bridges between Antarctic and South America, New Zealand and Australia. These could, however, have existed without seriously interfering with the mass relations of either continents or oceans.

I see no other acceptable means of getting crayfishes and their temnocephaloid parasites into South America, New Zealand, Australia and Madagascar, except by the

interposition of Antarctica, and have called this case "crucial." If the crayfishes traveled by this way, proving that a way was open, then all the probabilities are in favor of marsupials and hylid and leptodactylid frogs having traversed a similar path. The difficulties of detail can, I think, be solved by patient work, and it will be possible to explain or at least suggest why this creature did not get here, nor that creature there. But until it is definitely established and accepted that there was a habitable and inhabited Antarctica, with extensions to the other land masses of the southern hemisphere, the zoogeography of this hemisphere, or at any rate, of the Australasian region, must remain a matter of idle speculation. This much is certain—that, in the present state of knowledge, no hypothesis of northern dispersal can be reconciled with Australian facts.

Since the above was written, THE AMERICAN NATURALIST for July–August, 1925, has reached me, containing some further remarks by Dr. E. R. Dunn. Dunn does not seem to me to give adequate consideration to Metcalf's data, at least so far as concerns the hylids and leptodactylids. He is forced to surmise a geographical distribution for opalinids apart from that of their hosts, but does not appear to have considered the difficulty in which he lands himself as regards the distribution of the opalinid genera *Protopalina* and *Zelleriella*. These are confined to Australia and South America and are not found in Asiatic hylids and bufonids. How, then, does Dunn suppose that they reached Australia from South America, or, if he would prefer it so, *vice versa*? He complains twice that Metcalf has ignored the possibility of arboreal ranids having exterminated hylids in Africa and the Oriental region, but why should any one believe in, or worry about, such an improbable possibility? If some ranids have assumed arboreal adaptations, many Australian hylids have become terrestrial, and there is no evidence in either case that any other family has become exterminated in the process. If I may, for a moment

emulate Dunn's controversial style, he apparently holds, with Matthew, that because the Tertiary mammals could not enter Australia from the north, therefore the marsupials and the hylid and leptodactylid frogs did.

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SHORTER ARTICLES AND DISCUSSION

MENDELIAN INHERITANCE IN HYBRID WARBLERS

ANY attempt to explain the processes of evolution must take into account the Mendelian mechanism of inheritance and account for real or apparent changes in the constitution of genes. It is almost equally certain that an explanation of the mechanism of inheritance will have to consider the processes of evolution with reference to which that mechanism was evolved. But for the formulation of detailed hypotheses on the relation between genetics and evolution, there is needed a considerable body of information as to the genotypic relationships existing between the products of evolution, namely, valid wild species. In view of the difficulty of obtaining such information, at any rate in the animal kingdom, even a small bit of evidence presented by peculiarly favorable material may be worthy of note.

The Golden-winged Warbler, *Vermivora chrysoptera,* and the Blue-winged Warbler, *Vermivora pinus,* two related but clearly distinct wood-warblers of the eastern United States, are remarkable for the fact that in certain regions they interbreed, forming fertile hybrids which cross with one another and with the parent stocks. A sufficient number of observations on such crosses have been reported by observers in the field to provide data for the determination beyond reasonable doubt of a couple of factor pairs affecting plumage. The present paper is based on the observations collected in F. M. Chapman's "Warblers of North America."

Vermivora chrysoptera is characterized by gray upper parts, white under parts, yellow wing bars, and black throat and black auricular patch extending in a line through the eye. *V. pinus* has greenish upper parts, yellow under parts, white wing bars, and lacks the black markings, except for a narrow line through the eye.

The intermediate forms are described in the data by attribution to one of two hybrids, Lawrence's Warbler, *V. lawrencei,* and Brewster's Warbler, *V. leucobronchialis.* The wing bars, which are variable in both forms, are not described and so can not be considered here. *V. lawrencei* has the olive above and yellow below of *pinus,* and the black throat and auriculars of *chrysoptera. Leucobronchialis* has the gray upper parts and

white under parts of *chrysoptera,* but not the black markings. *Leucobronchialis* often shows some green on the back and yellow on the under parts, indicating the presence of complicating factors, but the *chrysoptera* body color is always clearly predominant.

From the above it would appear that we are dealing with two sets of characters: olive above and yellow below (yellow) as against gray above and white below (pale); and black throat and auriculars (black) as against absence of black markings (plain). The simplest assumption is that each represents a simple pair of allelomorphs. When the data are analyzed on this basis it is found that yellow (Y) is dominant over pale (y) and plain (B) over black (b) entirely consistent ratios being obtained throughout.

V. pinus, therefore, has the genotype Y ? B ?, *chrysoptera* yy bb, *leucobronchialis* yy B ? and *lawrencei* Y ? bb. The following is a transcription of the observations collected in Chapman, together with a determination of the doubtful genotypes where they are indicated by their relationships. An italicized letter in a parental genotype indicates that it must be present in one of the pair. Thus in (3) one or the other parent is almost certainly heterozygous for Y. The less certain determinations are in brackets.

ANALYSIS OF DATA IN E. M. CHAPMAN'S "WARBLERS OF NORTH AMERICA"[1]

(1) Y?B? × unknown = 1 Y?B? + 1 Y?bb
Y?Bb × ??b? = 1 Y?B? + 1 Y?bb

(2) yyB? × Y?B? = 3 Y?B?
yyB? × Y(Y)B(*B*) = 3 YyB?

(3) Y?B? × Y?bb = 6 Y?B?
YYBB × Y?bb = 6 Y?Bb

(4) yybb × unknown = 1 Y?B? + 1 yybb
yybb × YyBb = 1 YyBb + 1 yybb

(5) Y?B? × yybb = 1 Y?B?
Y?B? × yybb = 1 YyBb

(6) Y?B? × yyB? = 2 Y?B?
Y?B? × yyB? = 2 YyB?

(7) yyB? × unknown = 2 Y?B?
yyB? × Y??? = 2 YyB?

(8) yybb × Y?B? = 5 Y?B?
yybb × YYBB = 5 YyBb

(9) yyB? × yybb = "all" yyB?
yyB(B) × yybb = "all" yyBb

[1] 3d ed., 1917, p. 75.

(10) yyB? × yybb = more than one yyB? + "at least one" yybb
yyBb × yybb = more than one yyBb + "at least one" yybb
(11) Y?B? × yybb = "all" yyB?
YyB(B) × yybb = "all" yyBb

Here the expected ratio of yy in F_2 is only 50 per cent. Total number not stated.

(12) yybb × yyB? = 1 yyB? + 1 yybb
yybb × yyBb = 1 yyBb + 1 yybb

(1) Reported by J. Dwight, Jr., "Plumages and Molts of the Passerine Birds of New York," 1900, 246. (2) F. M. Chapman, "Additional Captures of *Helminthophila leucobronchialis*," *Auk*, IV, 1887, 348. (3) I. Bildersee, "Notes on the Nesting of Lawrence's Warbler," *Bird-Lore*, VI, 1904, 131; and C. W. Beebe, "Breeding of Lawrence's Warbler in New York City," *Auk*, XXI, 1904, 387. (4) A. K. Fisher, "Evidence concerning the Interbreeding of *Helminthophila chrysoptera* and *H. pinus*," *Auk*, 11, 1885, 359. (5) J. C. A. Meeker, "A Male Golden-winged Warbler mated with a Female Blue-winged Warbler," *Auk*, XXIII, 1906, 104. (6) E. H. Eames, "Notes on the Blue-winged Warbler and its Allies, in Connecticut," *Auk*, VI, 1889, 305. (7) L. B. Bishop, "*Helminthophila leucobronchialis* (breeding in Conn.)," *Auk*, XI, 1894 79. (8) J. H. Sage, "The Interbreeding of *Helminthophila pinus* and *H. chrysoptera*," *Auk*, VI, 1899, 299. (9), (10), (11), (12), W. Faxon, Mem. Mus. Comp. Zool., XL, No. 6, 1913, 311.

I now venture on what may appear unsupported speculation in order to indicate the type of problem which a sufficiently large body of related evidence may be called upon to solve.

Only one (*V. peregrinus*) of the seven other species of *Vermivora*, and that not closely resembling *pinus* or *chrysoptera* in other respects, has white under parts, all others being yellow or yellowish. Similarly, only *V. bachmani* has conspicuous black markings, a black cap and breast patch not including the auricular region or throat. If the usual generic characteristics are those of the common ancestor, B and Y would seem to be indicated as the ancestral and b and y as the divergent factors. In both cases the divergent or recent factor is recessive. It will be remembered that most *Drosophila* mutations are recessive. Again, the deviations from the wild and presumably original type of coat color in rats are all recessive. The testimony of *chrysoptera* tends to clear these well-known cases of the suspicion of being degenerative changes due to domestication and therefore not altogether comparable with mutations which take part in natural evolution. If it were found that recessive mutations (or apparent mutations) are the usual order of nature, it would be a very interesting conclusion. There is, by the way, another line of evidence pointing to some peculiar significance

of recessive factors in the process of evolution. At a certain stage in this development toward higher forms, plants and presumably animals changed from a state in which the soma is the haploid generation to one in which it is the diploid, and all organisms of any degree of complexity have this latter arrangement. We take it that there was some virtue in the change. Now this improvement can only have to do with recessive factors, since dominants affect a diploid soma precisely as they do a haploid.

A theory of evolution which is to apply to the plumages of birds must explain the intermittent recurrence of certain color patterns among species of different genera, families and even orders. External influences, including convergent natural selection, can here be pretty well ruled out, so that the explanation must lie in the mechanism of heredity and variation itself. In the case of *chrysoptera*, black throat and auriculars are found to be due to a change in a single gene or in linked genes. But many cases exist of species in which black throat or auricular appears independently, so that the inherited abilities to produce them are independent. Now if their presence in *chrysoptera* is due to two linked genes, each of these tendencies may be described as a tendency for a gene to mutate toward a factor for a certain black pattern. The integral gene remains as the sole seat of the hereditary constitution. But if in *chrysoptera* these two patterns are due to a single gene, that gene embodies the influence of two separable hereditary elements. In the latter contingency either there is a mechanism of what might be called potential heredity, which is not lodged in the genes at all, or the genes themselves may under unknown conditions be resolved into smaller hereditary units, as molecules are resolved into atoms.

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FURTHER OBSERVATIONS ON THE MANNER OF CLASPING THE HANDS

F. E. LUTZ, writing in this journal (Vol. XLII),[1] called attention to the curious fact that when the hands are clasped naturally with the fingers alternating, the same thumb is, for any given individual, usually outside. There is, according to my records, a very small percentage of individuals who clasp the hands with either thumb outside, but for the vast majority of people there is

[1] "The Inheritance of the Manner of Clasping the Hands."

only one comfortable way of doing this, namely, with either the right or left thumb uppermost. Lutz reported the thumb outside for about six hundred individuals. He found that 61 per cent. of the males and 58 per cent. of the females had the right thumb out. He states that the position assumed apparently has no relation to right or left-handedness and that no significant sexual dimorphism exists. But he obtained what he considered conclusive evidence that the mode of clasping the hands is inherited. Ultimately this curious phenomenon may, as Lutz suggested, be interpreted in the light of similar dispositions in lower animals. "Thus, the males of the common black cricket (Gryllus) usually keep the right tegmen over the left."

I wish here to report further observations concerning the position of the thumb in human beings. They suggest the need of further investigation both concerning the possibility of a causal connection between handedness types and the thumb outside and a difference in the sexes.[2]

Observations were recorded for 1,040 males and for 541 females—1,581 in all. 49.61 per cent. of the males placed the right thumb outside, and 54.16 per cent. of the females. The groups were not quite a random sampling but weighted unduly by the presence of a high proportion of ambidextral and left-handed individuals. Consequently the percentages of real value are those to be given in the next paragraph, which were obtained from individuals whose handedness type was recorded. Wherever it was possible information was obtained from the subjects of the above groups concerning their possession or not of lefthanded relatives. Their reports are not entitled to a high degree of confidence but are cited here as a matter of record. Three hundred and ninety-four men who placed the right thumb out reported 25.3 per cent. left-handed relatives; 393 men with the left thumb out reported 31.6 per cent. left-handed relatives. Two hundred and forty-three women with the right thumb out reported 36.2 per cent. left-handed relatives; 212 women with the left thumb out, 40.1 per cent. left-handed relatives.

To repeat, the percentages of real value are those that relate to individuals who have been classified with reference to handedness. These groups are somewhat smaller than those used above, but they represent much more detailed observation. Fifty-one per cent. of 572 right-handed men placed the right thumb

[2] These facts were gathered in a study of handedness made under a grant from the National Research Council through its Committee on Scientific Problems of Human Migration.

outside; 37.4 per cent. of 131 left-handed men placed the right thumb outside; 56.2 per cent. of 338 right-handed women placed the right thumb outside; 46.2 per cent. of 91 left-handed women placed the right thumb out.

Are these differences in percentages between the right-handed and the left-handed groups significant? To be significant a difference in percentages should be at least twice the standard deviation of the difference. The formula used to determine the standard deviation of a difference in percentages is the following:

$$\Sigma \cdot \text{dif} = \sqrt{\frac{p_1 q_1}{n_1} + \frac{p_2 q_2}{n_2}}$$

In this formula as applied here, n_1 represents the number of cases in the right-handed group and n_2 the number in the left-handed group. p_1 represents the percentage of right-handed individuals who are also right-thumbed and q_1 the percentage of right-handed individuals who are left-thumbed. p_2 and q_2 have the same indications for left-handed individuals. It appears from application of this formula that there does actually exist a difference between right-handed and left-handed men with respect to the likelihood of right or left thumb being uppermost. For the women the difference in percentages is not quite twice the standard deviation of the difference. Possibly, however, with larger groups a significant difference might be found.

In considering types of handedness it is, however, necessary to attempt refinement on the conventional division into right- and left-handedness. I have adopted the types of dextrality suggested by J. M. Rife in the *Psychological Review* for 1922. I have established handedness formulae for some fifteen hundred individuals and I have found his suggestions exceedingly fruitful. Rife's types of dextrality are determined by observation of the use of the hands in unimanual activities such as throwing or writing and bimanual activities such as batting, sweeping, spading and the like. In bimanual operations handedness is determined by the hand nearer the "business-end" of the implement. If the right (or left) hand is preferred in both unimanual and bimanual activities, the type is labeled RRR (LLL). If an individual is right-handed (or left-handed) in unimanual activities and left-handed (or right-handed) in bimanual activities the formula is RLL (LRR). If he is right-handed (or left-handed) for one-handed operations but with a divided preference for two handed ones, that is, right-handed (or left-handed) for opera-

tions high in the scale (batting) and left-handed (or right-handed) for operations low in the scale (sweeping), he is labeled RRL (LLR). To illustrate concretely, I may say that the typically RRR individual throws and writes right-handed, uses the baseball bat in the accepted right-handed fashion, and in sweeping places the right hand below the left on the broom-stick. The RLL individual throws and writes right-handed but bats over the left shoulder, placing the left hand above the right in so doing, and places the left hand below the right in sweeping. The RRL individual throws, writes and bats right-handed but places the left hand below the right in sweeping. The same three possibilities exist in the case of left-handed individuals who may be LLL, LRR or LLR in type. Certain intermediate or ambidextral types also occur as in the case of those individuals who change the relative position of the hands in batting or sweeping or who have a very mild unimanual preference. Into these sub-types I do not need to go at present, although I may say that apparently it is only in such ambidextral types that it is a matter of indifference as to which thumb is outside in clasping the hands.

In the table which follows percentages are given for the right thumb outside for each handedness type and for men and women separately.

	M		F	
Handedness	No.	% R Thumb Up	No.	% R Thumb Up
RRR	237	46.8	116	60.3
RRL	241	52.7	193	54.9
RLL	94	57.4	29	48.2
Total	572	51.04	338	56.2
LLL	31	32.3	34	47.1
LLR	37	43.2	33	48.5
LRR	63	36.5	24	41.8
Total	131	37.4	91	46.2

An inspection of this table suggests many interesting questions both with respect to significant differences in percentages for the right- and left-handed of the various handedness types and for the two sexes, but because of the small number in the groups of the left-handed no further statistical treatment will be attempted here. It is, however, obvious that a significant difference in percentages of right and left thumb up does occur for

males in the RLL and LRR groups and that some curious discrepancies occur in the percentages for the sexes, which, however, can not be adequately stated on the basis of the figures given in the table.

In comparison of my figures with those of Lutz, it is interesting to note that his unclassified group gives a higher percentage of right thumbs uppermost for both sexes than my figures show. There are several conjectural explanations for this difference. It may be due to the fact that his groups were smaller than mine. On the other hand, it is possible that his groups were more racially homogenous and more strongly right-handed than my groups. It may be that variation in the frequency with which dextrality types occur is a racial characteristic of some importance. Lutz's percentages were apparently obtained from Scotch subjects. Whether or not the Scotch are a strongly right-handed people, either by inheritance or habit, remains to be determined.

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CORRELATION AND MACHINE CALCULATION[1]

To the authors of the bulletin with the title above the criticism offered by Professor J. Arthur Harris[2] in the pages of this journal was decidedly surprising for two quite different reasons.

First: Readers of the article by Professor Harris would be left with a wholly inadequate and even erroneous concept of the objective and scope of this bulletin. The part concerning simple correlation, to which Professor Harris has apparently confined his attention, was quite incidental in the plan. It was included chiefly for the purpose of introducing the reader to the more complicated ideas, as well as methods of calculation, of the multiple and partial correlation coefficients. Of the six parts into which the bulletin is divided only a small part of the first one is devoted to a discussion of simple correlation. To quote from its introductory paragraphs:

> The present trend in all biological sciences, as well as in economics and psychology, is still further to extend the use of correlation, broadening its

[1] Wallace, H. A., and Snedecor, George W., "Correlation and Machine Calculation," Official Publication, Iowa State College of Agriculture and the Mechanic Arts, Vol. 23, No. 35, 1925.

[2] Harris, J. Arthur, "Correlation and Machine Calculation," AMERICAN NATURALIST, 59; 363–366, July-August, 1925.

scope to include the associations among more than two variables. One object of this bulletin is to present in simple, untechnical language some explanation of the meaning and uses of the various correlation coefficients, simple, partial and multiple. The second and principal object of the bulletin is to set forth explicit directions for the use of the usual commercial forms of calculating machines in finding correlation coefficients or related constants.

It is apparent that the main purpose of the bulletin was to put into available form a method for calculating and interpreting the constants of multiple correlation, a subject which was not touched upon by Professor Harris in his bibliography.

Second: Professor Harris has apparently assumed an unsympathetic attitude toward an effort by others to pursue his own practice of presenting to readers untrained in mathematical methods an easily understood interpretation of mathematical results, adequately illustrated. Professor Harris has a well-deserved reputation for writing about simple correlation and related concepts in such a way that the scientist can understand them without extensive courses in technical mathematics. The authors of "Correlation and Machine Calculation" have tried to advance the frontier in two directions, toward even greater simplicity and particularity in the instructions for calculation and toward the application of correlation to problems involving more than two variable characters. They have tried to help do for multiple correlation what Professor Harris himself has taken such an important part in doing for simple correlation. They frankly confess, therefore, a feeling of disappointment at the viewpoint which Professor Harris has adopted in his criticism.

In the matter of extent of bibliography cited, even the authors were not in entire agreement. The policy adopted was to refer the reader only to such passages as would furnish additional information upon some necessarily abbreviated discussion. The goal was brevity, simplicity and clarity. Doubtless the authors erred in some of their judgments. They respect the opinion expressed by Professor Harris in this matter. They still believe, however, that for a bulletin of this kind they pursued in the main a sound policy. For ordinary purposes, adequate bibliographies are available in all the texts and handbooks on the subject of mathematical statistics. It seemed superfluous, therefore, to encumber the pages of "Calculation and Machine Calculation" with material so easily found elsewhere.

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